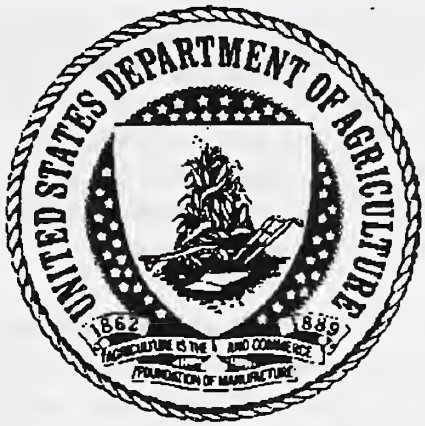
1996 PROGRESS REPORT ON FOOD SAFETY RESEARCH CONDUCTED BY ARS

Agricultural Research Service



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EXECUTIVE SUMMARY

This report documents the ARS research progress in 1996 to assure the safety of food products of animal origin. Highlights of the achievements are outlined below. These are further documented in the report along with many others.

Food safety starts with a healthy bird or animal that also is free of organisms which are pathogenic to humans. A patent was issued for the defined CF3™ culture which competitively inhibits *Salmonella* colonization in broiler chicks. Requirements for U.S. Food and Drug Administration approval are well along toward completion. The product has been sublicensed by the ARS CRADA partner to a Japanese company who is now selling it in Japan, and the product recently has been demonstrated to inhibit horizontal spread of *Salmonella gallinarum* which causes serious problems for the Mexican poultry industry.

Specific avian immune cytokines have been shown to induce a rapid and dynamic reaction in both newly hatched chicks and turkeys. This immune reaction results in the activation of heterophils which protects chickens against all *Salmonella* serotypes evaluated; including resistance to organ invasion. Development is being aided by the ARS CRADA partner.

Intimin, an outer membrane protein of *E. coli* O157:H7 encoded by the *eaeA* gene, was demonstrated to play an important role in the infection of newborn calves. This work identifies an important bacterial virulence factor and points to a possible means for preventing colonization and shedding in cattle. These approaches include production of high levels of intestinal antibodies directed against intimin or blocking of intestinal adherence of *E. coli* O157:H7 in cattle.

The typical increase in *Campylobacter* populations associated with the stresses of transport was prevented by feeding the yeast, *Saccharomyces boulardii*, at an elevated level for a brief period prior to feed withdrawal and transportation of broilers to the processing facility. Extending the use of this additive which has FDA GRAS status for some direct human uses, has great potential, as there is currently no other reported approach having this capability.

Ducks were infected with *Cryptosporidium parvum* oocysts recovered from cattle (an infective form), in order to determine if the parasite could be spread in their feces. It was demonstrated that the ducks could indeed release infectious oocysts in their feces two to seven days after ingesting the parasite. Thus, migratory birds may be a factor in the wide dissemination of the parasite in the environment.

In the **slaughter and processing arena** chemical antimicrobial treatment added a degree of food safety when beef tissue was stored as refrigerated vacuum packaged product for 21 days; and in no case did the treatments appear to offer any competitive advantage to select microorganisms, compared to untreated controls. Since beef consumption does not occur until some period of time after the carcass is processed, it is important to track pathogens well past the point of treatment when determining food safety advantages of specific treatments.

Marked *E. coli* O157:H7 was retrievable from the purge of raw beef combos (bulk lean trimmings) after 24 hours regardless of the location of the inoculated pieces of meat within the 75 cm meat column. Since the bacterial pathogen did migrate vertically downward into the purge of a beef combo, this vertical migration provides a means for representative sampling of the microbial content for this raw product.

Stationary phase (slow growing) *Listeria monocytogenes* cells had an extended lag period when transferred to a new growth environment, but the lag period was substantially reduced (that is, the cells more quickly began to grow rapidly) when log phase (rapidly growing) cells were used. This information helps provide exact estimates of how fast pathogens will grow under different conditions and is needed in order to design food processing and distribution systems with an adequate level of safety.

The acid tolerance of *E. coli* O157:H7 contributes to its ability to cause disease by increasing both its ability to persist in food, and its infectivity. Futher, acid tolerance also increases the pathogen's ability to withstand food safety control measures, such as, thermal processing and antimicrobial agents. To develop processing and control systems based on "worst case" situations, simple methods are needed for producing cells with maximum acid tolerance. ARS research (1) Developed a technique (overnight culturing in tryptic soy broth and dextrose) to induce maximum acid tolerance; and (2) Identified that the sensitivity to acid inactivation is dependent on acidulant identity (citric < lactic < acetic acid), prior exposure to an acid environment, and strain identity.

Residue detection focuses on methodology for drugs and environmental contaminants. Supercritical fluid extraction (SFE) which reduces solvent use was applied to the analysis of sulfonamides in eggs. The sulfonamides were readily extracted from eggs with a limit of detection of 0.025 ppm. The same FSIS laboratory carrying out SFE for sulfonamides in meat will now be able to perform these analyses on eggs.

Anti-halofuginone monoclonal antibodies were formatted as a competition enzyme-linked immunosorbent assay (cELISA) and found capable of detecting the drug in the 1 to 10 ppb range. The technology is being transferred to FSIS, and a private kit manufacturer is evaluating its use in a commercial immunoassay to serve as the rapid test requested by the drug manufacturer.

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(Pathogen Reduction and Residues in Food Products of Animal Origin)

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Part I. CONTROL OF FOODBORNE PATHOGENS IN LIVE ANIMALS

CONTROL SALMONELLA IN DOMESTIC ANIMALS

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OBJECTIVE A: Develop a cost effective means to prevent and/or control salmonellosis in poultry.

PROGRESS A: During the past year the defined CF3™ culture developed in our laboratory has been scaled up and maintained in a pilot plant facility in Madison, WI, owned and operated by our CRADA partner Milk Specialties Co., Dundee IL. The commercial culture is being produced by MS Biosciences, a new division of Milk Specialties Co., that was started in order to facilitate the commercialization of CF3™. The scaled up commercially prepared culture has been successful in reducing *Salmonella typhimurium* colonization in experimentally infected broiler chickens using a commercial spray application as the delivery method. Tests were performed with the commercial product in order to determine the minimal efficacious dose to be used in the first pivotal FDA approved clinical field studies. The first of the 5 eventual pivotal studies involving USDA and MS Bioscience scientists has been completed. A brief summary of the results of this 42 day grow-out FDA pivotal clinical challenge study are presented in Table 1. These data clearly document the efficiency of the CF3™ culture in reducing *Salmonella* contamination.

Table 1. Results of 42 day grow-out study.

Group	% *Salmonella* positive 21 days	% *Salmonella* positive 42 days
Controls	90	9
CF3™	23	0

IMPACT/TECH TRANSFER A: The patent for the defined CF3™ culture was issued on 12/26/95 and, the patent for the continuous-flow process is pending. During the past year the CF3™ culture was sublicensed by Milk Specialties Co. to Kyoritsu Shogi Co. of Japan. The patented CF3™ culture is presently maintained in commercial continuous-flow fermenters through a CRADA and by exclusive license to the BioScience Division of Milk Specialties Co., Dundee, IL. Currently, MS Bioscience is producing 200 million doses per year of which 50% has been contracted to the Japanese sub-licensee. The first product for commercial use was shipped to Japan in September of this year following experiments studying the horizontal transmission of Phage Type 4 *S. enteritidis* by Kyoritsu Shoji Co. demonstrating that CF3™ effectively blocked the spread of this pathogen. Presently, the CF3™ culture is in the process of obtaining FDA approval. Upon receiving FDA approval, this technology will be available to the United States poultry industry as an intervention strategy for control of human pathogens in poultry.

OBJECTIVE B: Test the efficacy of the patented ARS competitive exclusion culture, CF3™, on fowl typhoid *Salmonella* (*S. gallinarum).*

PROGRESS B: Fowl typhoid, a disease in poultry caused by *S. gallinarum,* causes large production (and therefore economic) losses to the poultry industry in developing nations. In cooperation with the Avian Disease Laboratory of The Universitaria Nacional of Mexico (UNAM), Mexico City, Mexico, a research partnership was formed in order to evaluate the efficacy of CF3™ to prevent or reduce colonization of this pathogen in chicks under commercially grown conditions. This research was a joint project that was planned and implemented by scientists at USDA-ARS Food Animal Protection Research Laboratory, College Station,TX; The Departemento de Produccion Animal:Aves, UNAM, Mexico City, Mexico; and Milk Specialties Biosciences Division, Dundee, IL. During a series of experiments in 1995–96, we demonstrated the ability of CF3™ to effectively control the horizontal spread of *S. gallinarum* and, therefore, reduce mortality due to this pathogen. A brief summary of these experiments is shown in Table 2.

Table 2. Effect of CF3™ on Mortality of Broiler Chicks Infected With 10^5 *Salmonella gallinarum.*

Group	% Seeder Chick Mortality	% Contact Chick Mortality
Controls	74.0	48.0
CF3™	7.5	3.8

CF3™ also decreased the horizontal spread of *S. gallinarum* in contact chicks infected with the pathogen.

IMPACT/TECH TRANSFER B: These experiments suggest that this product could have a large impact on the economics of poultry production in areas of the world where *S.*

gallinarum is endemic. The results from these experiments will be used in Mexico to seek regulatory approval.

OBJECTIVE C: Test the efficacy of CF3™ in colonization reduction by *Escherichia coli* O157:H7 in broiler chickens. The colonization of the gastrointestinal tract of poultry by this virulent human pathogen is of great concern to the poultry industry. Since *E. coli* O157:H7 is a gram-negative pathogen (like salmonellae) and can use the GI tract of food producing animals and poultry as a reservoir, in much the same manner that has been well documented for salmonellae, it was of interest to evaluate the efficacy of CF3™ in controlling colonization of this pathogen in commercial broiler chicks.

PROGRESS C: Experiments were performed to evaluate the concentration of *E. coli* O157:H7 needed to colonize the ceca of baby chicks and the affects of CF3™ on pathogen colonization. Results of these experiments are summarized in Table 3 and suggest that CF3™ effectively controls the colonization of *E. coli* O157:H7 in chicks.

Table 3. Effect of CF3™ on Colonization of Broiler Chicks by *Escherichia coli* O157:H7.

Log_{10} *E. coli* in chick cecae

Group	10^4 Challenge	10^6 Challenge	10^8 Challenge
Controls	2.04 +/- 1.05	1.86 +/- 1.29	6.23 +/- 0.72
CF3™	0.00 +/- 0.00	0.06 +/- 0.18	0.41 +/- 0.86

% *E. coli* positive cecae

Group	10^4 Challenge	10^6 Challenge	10^8 Challenge
Controls	85	75	100
CF3™	0	10	25

IMPACT/TECH TRANSFER C: These experiments suggest that treatment with CF3™ will result in significant protection against *E. coli* O157:H7 as-well-as both paratyphoid and typhoid *Salmonella*. This knowledge should further enhance the commercial feasibility of the defined culture.

OBJECTIVE D: Develop a model (based upon continuous-flow culture) of the poultry gastrointestinal eco-system and gain insights into the mode-of-action of competitive exclusion and provide scientific rationale for developing cultures for other food-producing animals. The development of models for *in vitro* scientific studies is important when developing cultures for large animals such as porcine and bovine where the cost of the

individual experimental unit is high. The use of such models will greatly reduce the cost of development of future defined cultures for large animals and enable this research to move forward quickly.

PROGRESS D: The CF3™ model has been used to measure the clearance rate of *S. typhimurium* and *Escherichia coli* O157:H7 from the culture at challenge levels from 10^1 to 10^6. The validity of the model has been confirmed by comparing the *in vitro* data to *in vivo* chick challenge data. Our data suggests that there is a theoretical limit to the ability of a competitive exclusion culture to protect against *Salmonella* and *E. coli* O157:H7 colonization. In the model, the culture is able to eliminate both of these pathogens at levels of up to 10^5/mL; however, at concentrations of 10^6, the CF model shows dramatic decreases in the concentration of both *Salmonella* and *E. coli* O157:H7, but fails to completely eliminate these pathogens. Another interesting aspect of this more basic research is the interactions found between *E. coli* O157:H7 and *S. typhimurium* in the CF-culture model. It appears that *E. coli* O157:H7 produces a molecule (bacteriocin colicin, etc.) that is extremely bacteriocidal against *S. typhimurium*. Additionally, as part of this research, monoclonal antibodies have been made that are specific to several of the CF3™ isolates, and these are now being used to study the effect of changes in environmental parameters such as nutrition and growth rate on culture profiles and efficacy. These parameters are based upon changes that can occur in the gastrointestinal system of poultry due to changes in diet or stress and should provide a model to predict how various environmental stresses influence the protective ability of the normal microbial flora against pathogen invasion. This research has been presented at several scientific meetings and, currently, a publication is in Journal submission.

IMPACT/TECH TRANSFER D: The expected impact of this technology will be the benefit of using models to develop and test competitive exclusion cultures thereby reducing the need for experimental animals historically required for this type of research. Additionally, models will provide a cost effective screening process for possible new cultures before testing in animals. This screening process will aide in culture development and should dramatically lower the cost of future defined culture development. The additional benefits of a better scientific understanding of the interactions between food pathogens and normal flora will have impact on a wide array of food pathogen research.

OBJECTIVE E: Develop a defined competitive exclusion culture to control *Salmonella* gut colonization in swine.

PROGRESS E: A competitive exclusion culture has been developed for swine using the same continuous-flow technique that was successful in developing CF3™, the defined competitive exclusion for poultry. This culture has been tested in a collaborative effort with Dr. Paula Cray at the NADC in Ames, IA, with whom we have established a cooperative research effort aimed at determining if the use of competitive exclusion in baby pigs is a viable intervention strategy to decrease *Salmonella* colonization in swine. In preliminary experiments prior to defining the microflora in the culture, excellent results

have been observed. In new born pigs receiving the porcine culture, total salmonellae was decreased in fecal swabs from 100% salmonellae positive in control pigs to 7% positive in pigs treated with the culture. Currently, by using the CF culture as a model, we are narrowing the population of bacteria in the culture in an effort to define a final efficacious culture. Initial contact with the FDA for an INADA (investigational new animal drug application) has been initiated to allow us to do on-the-farm testing of the product with a local producer.

IMPACT/TECH TRANSFER E: A CRADA has been formed with industry. As part of this CRADA, an industry supported research associate has been assigned to this project. The successful development of a defined competitive exclusion culture will provide swine producers with a new intervention strategy to aide in the control of salmonellosis in swine.

OBJECTIVE F: Reduce or prevent extraintestinal salmonellosis in neonatal poultry by modulating the innate immune response with avian cytokines.

PROGRESS F: Investigations to further develop a strategy for the modulation of the avian innate immune response as a means of preventing organ invasion of *Salmonella* in poultry have continued. Recent results from these experiments have shown that cytokines produced by T cells from chickens immunized against *S. enteritidis* (SE-ILK) confer protection to young chicks against both the paratyphoid and the typhoid salmonellae. In addition, we have shown that SE-ILK made from SE-immune chickens also will protect turkey poults from SE organ invasion. Interestingly, the administration of SE-ILK protects chickens against all *Salmonella* serotypes evaluated which is in marked contrast to most *Salmonella* vaccines. These findings suggest that the mechanism of host resistance to *Salmonella* in poultry may be less dependent on an antigen-specific response, but more contingent on initiating the correct innate host responses early during an infection. This hypothesis is in accord with our earlier studies showing that potentiation of the host inflammatory response by SE-ILK is associated with protection against paratyphoid *Salmonella* infections in poultry. In addition, investigations to further define the mechanism(s) of increased resistance to SE organ infectivity in chickens conferred by the prophylactic administration of SE-ILK also continued. We have found that: (a) the administration of SE-ILK and challenge with *Salmonella* results in a massive emigration of heterophils from the bone marrow into the blood stream, (b) production of an IL-8-like chemotactic protein in the local environment by the host in response to SE invasion which is responsible for the site-specific migration of heterophils to the site of bacterial. Additionally, at the time of the local IL-8 production, a population of peripheral blood heterophils begin to express IL-8 membrane receptors which are required for their continued migration to the inflamed site, (c) the heterophils from birds injected with SE-ILK have a significant enhancement (activation) of their biological effector functions including adherence, chemotaxis, phagocytosis, and bacterial killing, (d) a 10-fold increase in the expression of the heterophil-derived adhesion molecule, CD11b/CD18, which during the recruitment process, along with the activation of these phagocytic cells,

are essential for extravasation and sequestration of the heterophils at the inflamed site, (e) a massive influx of the activated inflammatory heterophils from the blood to the site of infection by *Salmonella*. Thus, as seen here and in our previous experiments, the administration of ILK induces a rapid and dynamic reaction in the bird that results in the activation of heterophils with a subsequent resistance to systemic *Salmonella* infections. In conclusion, our studies indicate that ILK contains specific cytokines that are important mediators of leukocyte recruitment and activation in avian salmonellosis. Immunologic manipulation (preventive activation) of avian innate resistance may serve as an important industry applicable method for the prevention and control of intestinal and organ colonization by paratyphoid and typhoid salmonellae infections in broiler and table-egg producing flocks.

IMPACT/TECH TRANSFER F: The administration of immune cytokines induces a rapid and dynamic reaction in both neonatal chicks and turkeys that results in the activation of heterophils with a resultant resistance to intestinal and organ colonization by both paratyphoid and typhoid salmonellae. A CRADA with Embrex to administer immune cytokines to chicks *in ovo* continued.

PUBLICATIONS:

Corrier, D. E., D. J. Nisbet, B. M. Hargis, P. S. Holt, and J. R. DeLoach. 1996. Provision of lactose to molting hens enhances resistance to *Salmonella enteritidis* colonization. *J. Food Prot.* (In press).

Corrier, D. E., D. J. Nisbet, N. K. Keith, E. D. Dunlap, and J. R. DeLoach. 1996. Spray application and dosage titration of characterized CF3 competitive exclusion culture for *Salmonella* control in broiler chickens, pp 272–275. Proceedings of the 45th Annual Meeting of the Western Poultry Disease Conference, Cancun, Mexico.

Hume, M. E., D. E. Corrier, D. J. Nisbet, and J. R. DeLoach. 1996. Reduction of *Salmonella* crop and cecal colonization by a characterized competitive exclusion culture in broilers during growout. *J. Food Prot.* 59:688–693.

Hume, M. E., A. G. Hollister, D. J. Nisbet, D. E. Corrier, and J. R. DeLoach. 1996. Effect of a characterized continuous-flow culture of cecal bacteria on *Salmonella typhimurium* crop colonization in broiler chicks. *Avian Dis.* **40**:391–397.

Kogut, M. H., V. K. Lowry, R. B. Moyes, L. L. Bowden, R. Bowden, K. Genovese, and J. R. DeLoach. 1996. Lymphokine-augmented activation of avian heterophils. *Poult. Sci.* (In press).

Kogut, M. H., E. D. McGruder, B. M. Hargis, D. E. Corrier, and J. R. DeLoach. 1996. Role for IL-8 in the accumulation of peritoneal heterophils in chicks following the intraperitoneal administration of *Salmonella enteritidis*-immune lymphokines and *Salmonella enteritidis*. *Poult. Sci.* (In press).

Kogut, M. H., R. B. Moyes, and J. R. DeLoach. 1996. Granulocyte-colony stimulating factor mediates the *Salmonella enteritidis*-immune lymphokine potentiation of the acute avian inflammatory response. *Inflammation* (In press).

Kogut, M. H., G. I. Tellez, E .D. McGruder, R. A. Wong, A. Isibasi, V. N Ortiz, B. M. Hargis, and J. R. DeLoach. 1996. Evaluation of *Salmonella enteritidis*-immune lymphokines on host resistance to *Salmonella gallinarum* infection in broiler chicks. *Avian Pathol.* (In press).

McGruder, E. D., M. H. Kogut, D. E. Corrier, J. R. DeLoach, and B. M. Hargis. 1995. Characterization of colony-stimulating activity in the avian T cell-derived factor, *Salmonella enteritidis*-immune lymphokine. *Res. Vet. Sci.* **60**:222–227.

Nisbet, D. J., D. E. Corrier, S. C. Ricke, M. E. Hume, J. A. Byrd, and J. R. DeLoach. 1996. Maintenance of a competitive exclusion culture by continuous-flow fermentation. *J. Food Prot.* (In press).

Nisbet, D. J., D. E. Corrier, S. C., Ricke, M. E. Hume, and J. R. DeLoach. 1996. Maintenance of a competitive exclusion culture by continuous-flow fermentation, pp 275–279. Proceedings of the 45th Annual Meeting of the Western Poultry Disease Conference, Cancun, Mexico.

Ziprin, R. L. 1996. Efficacy of two avian *Salmonella*-immune lymphokines against liver invasion in chickens by *Salmonella* serovars with differing O-group antigens. *Avian Dis.* (In press).

Ziprin, R. L. 1996. Heterophil response to intraperitoneal challenge with invasion-deficient *Salmonella enteritidis* and *Salmonella*-immune lymphokines. *Avian Dis.* (In press).

Ziprin, R. L., M. H. Kogut, E. D. McGruder, B. M. Hargis, and J. R. DeLoach. 1995. Efficacy of *Salmonella enteritidis*-immune lymphokines from chickens and turkeys on SE organ invasion in day-old chicks and turkey poults. *Avian Dis.* **40**:186–192.

PATHOGENESIS, DETECTION, AND CONTROL OF *SALMONELLA ENTERITIDIS* AND OTHER SALMONELLAE IN CHICKENS

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OBJECTIVE A: Develop and evaluate sensitive and efficient methods for testing chickens and eggs for *Salmonella enteritidis*.

PROGRESS A: We determined that testing for specific antibodies in the yolks of eggs laid by hens experimentally infected with *Salmonella enteritidis* (SE) predicted the likelihood that contaminated eggs would be produced by these hens with a sensitivity and specificity that matched or exceeded results obtained by culturing voided feces. SE contamination of eggs has been a significant source of human illness in recent years. Bacteriological culturing to detect SE in eggs is an important aspect of programs to reduce the likelihood that contaminated eggs will reach consumers, but egg culturing is slow and labor intensive. Flocks are thus generally subjected to preliminary screening tests, which should ideally be able to detect infection with high sensitivity and to consistently predict the probability of egg contamination. We experimentally infected hens with SE and collected their eggs to culture for SE in the contents. Eggs from each hen were tested for the presence of specific yolk antibodies using two enzyme immunoassay methods. Samples of voided feces also were collected from each bird and cultured for SE. Although the shedding of SE in feces decreased over time and egg yolk antibodies were more easily found in the later stages of the experiment, both of these types of tests were determined to be useful for predicting whether particular hens would lay contaminated eggs. Over 80% of the hens that laid SE contaminated eggs were detected as infected by fecal culturing and over 90% were detected by one of the assays for egg yolk antibodies.

IMPACT/TECH TRANSFER A: This research demonstrated to regulatory officials and diagnostic laboratories that egg yolk antibody testing allows rapid and effective screening to identify SE infected laying flocks using easily collected samples.

OBJECTIVE B: Examine the horizontal transmission of *Salmonella enteritidis* in hens undergoing a fast.

PROGRESS B: Feed removal is a prevalent method used by the layer industry to induce a second egg cycle from aging flocks in declining lay. We found earlier that fasted hens were more susceptible to an infection by *Salmonella enteritidis* (SE) and that the

organism disseminated rapidly through a group of fasted hens compared with minimal transmission in fed hens. The normal route of infection for SE is the oral-fecal route — that is, through the consumption of fecally-contaminated materials. Therefore, direct contact with infected birds or their feces was thought necessary for infection. However, current experiments showed that fasted hens could become infected with SE originating from hens in distant cages. Bacteriological monitoring of the room air found progressively more SE during the course of the experiment which corresponded with the high numbers of SE released by fasted infected hens and the expanding numbers of SE on the floor beneath the cages.

IMPACT/TECH TRANSFER B: These studies provide evidence for an alternative route of infection of hens by SE under certain stress conditions and indicate that a major industry practice may provide this stress. The results of these studies were presented in May at the national meeting of the American Society for Microbiology and were selected by the Society for release to the news media as information of potential interest to other scientists and to the general population at large. The information was released by the national wire services and also appeared on the news channel CNN.

OBJECTIVE C: Identify conditions that induce the expression of virulence genes by *Salmonella enteritidis*.

PROGRESS C: Virulent strains of *Salmonella enteritidis* (SE) have cell-surface characteristics that include production of high-molecular-weight (HMW) lipopolysaccharide and hyperflagellation. Previously, it was shown that these characteristics rather than phage type are associated with high levels of organ invasion and egg contamination. *S. pullorum* is an avian-adapted organism that provides a stringent control for these studies since it has been classified as an aflagellate organism ever since its isolation nearly 100 years ago, and it also is a pathogen that reliably contaminates eggs. Results show that a particular combination of conditions are required to induce flagellation by *S. pullorum*, which can be demonstrated by scanning electron microscopy, polyacrylamide gel electrophoresis, and slide agglutination reactivity with flagellin detecting H-antiserum. Strain differences were apparent and indicated that some strains undergo more complete differentiation under a broader range of conditions. These results indicated that the genetic potential of *Salmonella* is not realized under laboratory conditions, and that this pathogen's physiological capabilities in certain environments are much greater than appreciated.

IMPACT/TECH TRANSFER C: This research resulted in the patent application No. 08/649,501 entitled "A Complex Growth Supplement for Maintenance of Bacterial Cell Viability and Bacterial Cell Differentiation," a CRADA with Maine Biological Laboratories, Waterville, ME, and a specific research agreement with Stanford University.

OBJECTIVE D: Determine if phage type 4 strains of *Salmonella enteritidis* exhibit similar within phage-type heterogeneity in virulence attributes as seen in phage types 8 and 13a.

PROGRESS D: Techniques developed to detect within phage type (PT) heterogeneity of *Salmonella enteritidis* (SE) were used to detect those strains of PT 4 SE that are more virulent in mice and more invasive in chickens. Virulent isolates of PT 4 SE were more tolerant to heat, acid, and hydrogen peroxide and survived longer in aerosols in comparison to avirulent strains.

IMPACT/TECH TRANSFER D: This research was part of an international collaboration between researchers from England, Australia and the United States and led to published recognition that simple assessments of virulence within phage type are possible. These techniques are currently being applied in Britain to study the epidemiology of PT-DT104 *Typhimurium*, which is causing 3% mortality in people who contract this illness.

OBJECTIVE E: Assess whether phage type 4 *Salmonella enteritidis* strains represent a more serious threat to public health and to poultry than strains of phage types more commonly found in the U.S.

PROGRESS E: We determined that, although significant differences were sometimes observed between individual *Salmonella enteritidis* (SE) isolates in the frequencies at which they colonized the intestinal tracts and invaded to reach the spleens of inoculated chicks, no consistent overall pattern differentiated phage type 4 isolates from isolates of other phage types. SE contamination of eggs is an important public health problem in the U.S. and in many other nations. In the U.S., SE strains of a variety of phage types have been associated with egg-transmitted illness. In Europe, however, nearly all SE isolates from both humans and poultry during the past decade have been phage type (PT) 4. Although previously reported only rarely, PT4 SE strains have recently been isolated from both poultry and humans in the U.S. The widespread prevalence of PT4 strains in European poultry raises questions about whether PT4 SE strains might be more infectious for chickens than other PT's and if control strategies for SE in U.S. poultry need to be revised to account for the presence of PT4. We evaluated the ability of SE isolates of various phage types found in the U.S. (including PT4) to colonize the intestinal tract and invade to reach internal organs in experimentally infected chicks. Groups of chicks were infected with various oral doses of three PT4 isolates and three isolates of other PT's. Although significant differences in the ability to infect chicks were observed between individual SE isolates, no overall pattern of differences was evident between PT4 isolates and isolates of other PT's.

IMPACT/TECH TRANSFER E: This demonstrated to regulatory officials and the poultry industry that strategies developed for controlling other SE PT's should still be applicable if PT4 becomes more prevalent in the U.S.

PUBLICATIONS:

Arnold, J. W. and Holt, P. S. 1996. Cytotoxicity in chicken alimentary secretions as measured by a derivative of the tumor necrosis factor assay. *Poult. Sci.* 75:329–334.

Gast, R. K. and S. T. Benson. 1995. The comparative virulence for chicks of *Salmonella enteritidis* phage type 4 isolates and isolates of phage types commonly found in poultry in the United States. *Avian Dis.* 39:567–574.

Guard-Petter, J., L. H. Keller, M. Mahbubur Rahman, R. W. Carlson, and S. Silvers. 1996. A novel relationship between O-antigen variation, matrix formation, and invasiveness of *Salmonella enteritidis*. *Epidemiol. Infect.* (In press).

Holt, P. S., Stone, H. D., Gast, R. K, and Porter, R. E., Jr. 1996. Growth of *Salmonella enteritidis* (SE) in egg contents from hens vaccinated with an SE bacterin. *Food Microbiol.* (In press).

Humphrey, T. J., A. Williams, K. McAlpine, M. S. Lever, J. Guard-Petter and J. M. Cox. 1996. Isolates of *Salmonella enterica Enteritidis* PT4 with enhanced heat and acid tolerance are more virulent in mice and more invasive in chickens. *Epidemiol. Infect.* (In press).

CONTROL OF *SALMONELLA* IN DOMESTIC ANIMALS

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OBJECTIVE A: Develop control procedures to prevent colonization of chickens and/or turkeys with competitive exclusion cultures and bacterial disinfection within the hatching cabinets.

PROGRESS A: Our patented competitive exclusion culture, now called Mucosal Starter Culture (MSC) which has been licensed by the Continental Grain Company is undergoing trials designed to secure FDA approval. A new method to store and distribute the product has been developed, and we are currently conducting large scale efficacy trials, dose titration trials, and target animal safety trials. Maximum efficiency of MSC treatments will be gained if *Salmonella* are controlled in the hatching cabinets. We challenged day-of-hatch chicks either by mouth, cloaca, or eye and nasal passage to determine the importance of *Salmonella* transmission in the hatching cabinet. We observed that each route of challenge resulted in bird infection. These seeder birds were then capable of transmitting *Salmonella* during handling, transportation, and after placement on the farm. This research emphasizes the need to develop salmonellae control procedures for breeder flocks, hatcheries, broiler houses, and during transportation. In one of these studies, we were able to obtain a 99.9% reduction of eggshell bacteria by sanitizing eggs in the transport buggy at the hatchery with a quaternary ammonium solution spray.

IMPACT/TECH TRANSFER A: Two patents have been issued and licensed by commercial companies. The MSC process patent has been licensed by Continental Grain Co., Duluth, GA and they anticipate having an FDA approved product on the market within the next year. *In ovo* application of bacterial cultures has been licensed by the EMBREX Inc., Raleigh, NC. Studies are currently being conducted to determine the optimal culture to be used *in ovo*. A new CRADA with the Continental Grain Company is being developed. Cooperative hatchery and hatchery cabinet disinfection research studies with Three Wayne Farm hatcheries in Georgia, Alabama, and Arkansas is anticipated this year.

OBJECTIVE B: Develop control procedures to prevent *Salmonella* colonization in chickens and/or turkeys with the yeast *Saccharomyces boulardii*.

PROGRESS B: We have been investigating the ability of *Saccharomyces boulardii* to reduce *Salmonella* colonization of poultry when administered by a variety of methods. Feeding the yeast at an elevated level for a brief period of time prior to transporting the birds to the processing facility successfully prevented the typical increase in *Salmonella* populations associated with the stresses of transport. *In ovo* application of yeast was successful in reducing the number of chicks colonized with *Salmonella*. A combined treatment of yeast and an antibiotic delivered *in ovo* has been shown to be more effective than either treatment alone. We are currently working to adapt this technology to a commercial *in ovo* injection system.

We also have demonstrated that yeast delivered in the feed throughout grow-out can reduce the number of chickens colonized with *Salmonella*. We are currently conducting trials to determine how combinations of various yeast treatments and other intervention methods such as Mucosal Starter Cultures may interact to further reduce *Salmonella* colonization of poultry.

IMPACT/TECH TRANSFER B: Two patents have been written, and a Cooperative Research and Development Agreement is in effect with the Lallemand Co. (Montreal, Canada) who has licensed this patent pending technology. The potential impact associated with the treatment of humans, having antibiotic associated diarrhea, with *S. boulardii* is tremendous, particularly in preventing transport stress related spiking of *Salmonella*. Currently, we know of no other technologies that have been successful in reducing transport related proliferation of *Salmonella*.

OBJECTIVE C: To determine the efficacy of bacterial cultures to reduce intestinal numbers of *Clostridium perfringens* (Cp) in chickens.

PROGRESS C: *Clostridium perfringens* (Cp) is the third most common known cause of food poisoning in humans and necrotic enteritis in chickens. We are developing chicken colonization and disease models so that intervention procedures can be developed and evaluated. After challenging chicks on corn-based diets with high numbers of Cp, the Cp count in the intestine declined with increased age of birds. When birds were on a rye diet and were challenged with high or low numbers of Cp, the initial count was high and remained at similar levels or increased with age of bird. Two cultures developed in our lab and patented for use in reducing salmonellae numbers in chickens were evaluated for their ability to reduce Cp in the chicks. Both a mucosal starter culture and a *Saccharomyces cerevisiae boulardii* yeast culture reduced Cp counts, and levels were sometimes reduced 5–6 log factors so that no Cp were detected in treated chicks. A defined culture developed in our laboratory and consisting of identified bacterial strains isolated from the intestine of adult birds also reduced Cp numbers and frequency. Based on these promising results, the cultures will be evaluated in floor pen experiments and field trials for their ability to reduce numbers of naturally occurring Cp.

IMPACT/TECH TRANSFER C: The economic impact of *C. perfringens* related necrotic enteritis as a production problem in the poultry industry is increasing and is well documented. *C. perfringens* transmitted from poultry are sometimes associated with human diarrhea. Prior to this research, no models had been developed or were being used for studying this organism in chickens. The yeast *Saccharomyces boulardii* is used to treat antibiotic associated diarrhea in humans and the mucosal starter culture is expected to receive FOA approval in FY-97. If effective, these intervention procedures could reduce necrotic enteritis in poultry production and at the same time reduce human exposure to Cp.

OBJECTIVE D: Develop and test efficacy of mucosal competitive exclusion (MCE) technology to prevent *Salmonella* colonization of porcine and *E. coli* O157:H7 in bovine.

PROGRESS D: For development of both pig and cow MCE cultures, we have used similar technology to that utilized in the successful Mucosal Starter Cultures for poultry. The efficacy of these cultures is being studied by Dr. Paula Cray at NADC who is conducting pen trials with pigs using this culture to protect against *Salmonella* colonization. In two trials, she has achieved significant reductions in both the frequency and levels of *Salmonella* in young pigs. In late August, pen trials began with Kansas State University to test the effectiveness of cow MCE against *E. coli* O157:H7 in calves. We anticipate continuing to cooperate with scientists at Ames and Kansas State in both of these project area's.

IMPACT/TECH TRANSFER D: The economic impact of porcine *Salmonella* and bovine *E. coli* O157:H7 is enormous. The preliminary results of this technology which has been successful in helping to control *Salmonella* in poultry were very encouraging. The ARS patent review committee has determined that a patent should be written. This patent application is being developed and should be submitted to the patent office in September, 1996.

OBJECTIVE E: To determine the mechanism of attachment of *Salmonella* to chicken intestinal mucus and poultry skin, and to identify intestinal bacterial isolates that inhibit attachment.

PROGRESS E: Preexposure of mucus to calcium enhanced subsequent attachment of *S. typhimurium* (St) cells to the mucus after a washing step. But, preexposure of St cells to calcium had no effect. Calcium may bind to some component of mucus to increase attachment of St cells by a conformational change or by bridging the distance between negative charges on the bacterial cell and the mucus. Exposure of mucus to washed cells or culture supernatant of *lactobacilli* strains isolated from the chicken intestine inhibited subsequent attachment of St cells to the mucus, and the inhibitory effect was enhanced by preheating cells or supernatant at 80 °C. Both gamma-irradiated and formaldehyde-treated St cells attached like live cells to poultry skin. St cells which were grown in

different media had different cell surface charges but attached to skin in a similar manner. Results suggest that *de novo* synthesis of attachment molecules or cell charge were not related to attachment.

IMPACT/TECH TRANSFER E: Strains of bacteria that block attachment of salmonellae to the mucosal layer of the chicken intestinal tract may be useful when fed to chicks in preventing subsequent intestinal colonization by *Salmonella*. An increased understanding of the mechanisms of attachment of *Salmonella* to poultry skin could provide information that can be used to prevent attachment or to induce detachment of cells of this pathogen. The cooperator in this project, The University of Georgia, Food Science Department, is continuing research in this area to follow-up on results obtained through a cooperative agreement.

OBJECTIVE F: Determine the primary sources of *Listeria monocytogenes* in an integrated broiler chicken operation.

PROGRESS F: *Listeria monocytogenes* were found on less than 4% of the samples taken from commercial broiler hatcheries and only once from 230 samples of whole bird rinses (feathers on) and cecal samples. After processing, over 25% of the broiler carcasses were positive for *L. monocytogenes*. This suggests that *Listeria* is coming into the processing plant sporadically on live birds and then creating a reservoir in the plant which leads to cross-contamination during processing.

IMPACT/TECH TRANSFER F: By confirming that poultry contamination with *Listeria monocytogenes* is primarily a processing plant problem, industry and researchers can concentrate research and resources on developing procedures to prevent build-up and cross-contamination of products in the processing plant.

OBJECTIVE G: Evaluate and develop more rapid and effective methods for recovery of bacterial pathogens from poultry and other food products.

PROGRESS G: The DuPont BAX PCR based *Salmonella* detection system was evaluated with poultry meat and environmental samples. Greater than a 95% correlation with conventional procedures was found. The DuPont Automated RiboPrinter system was used to evaluate *Salmonella* isolates from a commercial turkey field trial study. Isolates were compared to the APHIS National Serotyping Laboratory. Again, greater than 95% of the isolates were identified the same as with the APHIS laboratory. Refinement of the Universal Preenrichment Medium developed in this laboratory to allow detection of different pathogens by ELISA, probe or PCR technology continues.

IMPACT/TECH TRANSFER G: The BAX PCR study was the first in the world using this PCR technology to identify *Salmonella* from poultry products. As a result of the

RiboPrinter study, we are hoping to purchase a RiboPrinter to use in our proposed nationwide *Salmonella* epidemiology study. The patent for Universal Preenrichment broth ("Preenrichment broth medium for the simultaneous sampling of foods for *Salmonella* and *Listeria*", patent number 5,145,786) has been licensed by DIFCO (Detroit, MI) and commercial sales for the broth continue to grow.

PUBLICATIONS:

Bailey, J. S., R. J. Buhr, N. A. Cox, and M. E. Berrang. 1996. Effect of hatcher cabinet sanitation treatments on *Salmonella* cross-contamination and hatchability of broiler eggs. *Poult. Sci.* **75**:191–196.

Bailey, J. S. and N. A. Cox. 1995. Hatchery and breeder flock contamination, pp 45–51. Proceedings of the Georgia Poultry Conference, Gainesville, GA.

Berrang, M. E., N. A. Cox, J. S. Bailey, and R. J. Buhr. 1995. Efficacy of ultra-violet light for elimination of *Salmonella* on broiler hatching eggs. *J. Appl. Poult. Res.* **4**:422–429.

Cosby, D. E. 1996. Isolation of bacteria from the chicken crop and ceca which produce inhibitory activity against *Salmonella typhimurium*. Master's Thesis. Department of Food Science and Technology, University of Georgia, Athens, GA.

Cox, N. A. and J. S. Bailey. 1995. Reducing bacteria from farm through processing, pp 28–31. Proceedings of the 30th National Meeting on Poultry Health and Processing, Ocean City, MD.

Cox, N. A., J. S. Bailey, and N. J. Stern. 1995. Research efforts to intervene in the *Salmonella* colonization of broiler chicks, pp 193–199. Proceedings of the XII European Symposium on the quality of poultry meat, Glasgow, Scottland.

Craven, S. E. 1995. Increased susceptibility of *Salmonella typhimurium* to hydrophobic antibiotics in the presence of outer-membrane-disorganizing agents: effect on the intestinal colonization of broiler chicks. *J. Appl. Poult. Res.* **4**:333–340.

Craven, S. E., K. Y. Kim, and D. D. Williams. 1995. Inhibition of *Salmonella typhimurium* attachment to and growth in cecal mucus by chicken bacterial isolate, pp 77–87s. Proceedings of the XII European Symposium on the Quality of Poultry Meat, Zaragoza, Spain.

Kim, K. Y., J. F. Frank, and S. E. Craven. 1996. Attachment of *Salmonella typhimurium* on modified poultry skin surface. *J. Food Sci.* **61**:442–443.

Kim, K. Y., J. F. Frank, and S. E. Craven. 1996. Three-dimensional visualization of *Salmonella* attachment to poultry skin using confocal scanning laser microscopy. *Lett. Appl. Microbiol.* **22**:280–282.

Kim, K. Y., H. S. Lillard, J. F. Frank, and S. E. Craven. 1996. Attachment of *Salmonella typhimurium* to poultry skin as related to cell viability. *J. Food Sci.* **61**:439–441, 448.

Line, J. E., N. A. Cox, N. J. Stern, and J. S. Bailey. 1995. In *ovo* yeast treatment to diminish *Salmonella* populations in poultry. Patent Pending (S.N. 08/542,488).

Line, J. E., N. A. Cox, N. J. Stern, J. S. Bailey, C. Ricks, P. Phelps, and M. Knight. 1996. In *ovo* antibiotic and microbial treatment to diminish salmonellae populations in avians. Patent Pending (S.N. 08/671,743).

Russell, S. M., D. L. Fletcher, and N. A. Cox. 1995. Comparison of media for determining temperature abuse of fresh broiler carcasses using impedance microbiology. *J Food Prot.* **58**:1124–1128.

Russell, S. M., D. L. Fletcher, and N. A. Cox. 1996. The effect of temperature mishandling at various times during storage on detection of temperature abuse of fresh broiler chicken carcasses. *Poult. Sci.* **75**:261–264.

Russell, S. M., D. L. Fletcher, and N. A. Cox. 1996. Spoilage bacteria of fresh broiler chicken carcasses. *Poult. Sci.* **75**:2041–2047.

Williams, D. D. 1996. Inhibition of *in vitro* attachment of *Salmonella typhimurium* to chicken intestinal mucus by native chicken microflora. Master's Thesis. Department of Food Science and Animal Industries, Alabama A and M University, Huntsville, AL.

PATHOGENESIS, TRANSMISSION, AND CONTROL OF SALMONELLOSIS IN SWINE

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OBJECTIVE A: Identify virulence factors important in the pathogenesis of *Salmonella choleraesuis* and *Salmonella typhimurium* in swine, including antigens which may be important in eliciting an immune response.

PROGRESS A: An ELISA designated SalAD (*Salmonella* Antigen Detection) was produced in our laboratory using a mixed ELISA format with antigens prepared from 4 serogroups (B, C1, D1, E1). The sensitivity was tested against sera from experimentally infected, long-term carrier swine and known infected swine herds. The specificity of the test was evaluated using sera from swine free of *Salmonella* spp., swine which had been infected but cleared the bacteria, and swine infected with *E. coli* or *Yersinia enterocolitica* (negative for *Salmonella* spp.). Results indicate that the sensitivity and specificity are > 95% and > 85%, respectively. False positives were not obtained from pigs infected or vaccinated with *E. coli*. When compared to the Danish MIX-ELISA (a lipopolysaccharide antigen based system), our SalAD had a higher specificity. The SalAD is a useful test for monitoring the presence of *Salmonella* antigen(s) in swine herds. Currently it is being used to monitor the immune response in pigs from farrow to finish in order to assess when exposure to *Salmonella* may first occur.

IMPACT/TECH TRANSFER A: Information regarding detection of carrier animals will benefit those seeking to implement HACCP plans. Assessment of the immune response on the farm will provide researchers with a better understanding of the timeframe in which pigs are exposed to *Salmonella*. This work has been presented to commodity groups and professionals at scientific meetings.

OBJECTIVE B: Define the epidemiology and transmission of *Salmonella* in swine.

PROGRESS B: Previously, we esophagotomized (surgical procedure that prevents oral exposure) pigs and demonstrated that the gut tissues were positive within 3 hrs post-challenge by intranasal instillation of *S. typhimurium*. We hypothesized that the inverse (challenge into the gut bypassing the tonsil and nasopharyngeal tissues) would not produce positive tissues other than in the gut. Gelatin capsules containing feed and 109 CFU *S.*

typhimurium were dried to hardness. Pigs were esophagotomized and the capsule was introduced into the esophagus and pushed through the cardia into the stomach. Necropsies were conducted at 3 and 22 hrs post-challenge. At 3 hrs the stomach contents were positive in 3/4 of the pigs and the only other tissues that were positive were the mid-ileum and cecal contents of one pig. (The distal portion of the esophagus closest to the cardia was positive in 3/4 of the pigs.) At 22 hrs, only 1/3 of the pigs had positive tissues in locations other than the gut (tonsil, lung, bronchiole lymph node, and spleen). However, 2/3 of the pigs had quantifiable levels of *Salmonella* in the cecal contents, ileocolic junction, ileocolic lymph nodes which was not seen when pigs were challenged intranasally. Interestingly, *Salmonella* was not recovered from the stomach at 2 hrs. These data confirm that an intranasal challenge produces wider tissue dissemination than a direct stomach challenge, but challenge into the stomach produces higher numbers in the gut tissues.

Epidemiology of *Salmonella* spp. — The USDA:APHIS:VS conducted both a pilot and a large study of *Salmonella* spp. in swine herds as part of the NAHMS Swine 95 survey. Fifty fresh fecal samples (25 g/sample) were collected from finisher age pigs from 15 herds for the pilot study. One gram of feces from each sample was placed into each of two culture media; tetrathionate broth (Tet) or GN Hajna broth (GN) followed by subculture into Rappaport R-10 medium (GN-R and Tet-R). All Tet, GN-R, and Tet-R were struck onto brilliant green agar with sulfadiazine (BGS); Tet also was struck onto xylose-lysine-Tergitol 4 (XLT4) and brilliant green agar with novobiocin (BGN) plates. The sample and herd prevalence rates were 3.8% (27/715 positive) and 40% (6/15 positive), respectively. The predominant serotype identified was *S. derby* (93%; 25/27) followed by *S. infantis* (3.7%; 1/27) and *S. untypable* (3.7%; 1/27). *Salmonella* spp. were most often recovered from samples cultured in Tet-R (93%). Culture in GN-R was least sensitive. Identification of positive herds was best achieved by culture in Tet-R (83.3%) when compared to either Tet alone (50%) or GN-R (33.3%). Additionally, culture in Tet-R was most likely to identify all positive samples within a herd. No differences between plating media (BGS, BGN, and XLT4) were observed. These data provide information on the status of *Salmonella* spp. from finisher age swine and serve as baseline information for the larger Swine 95 survey. Additionally, these data demonstrate that *Salmonella* spp. have a higher probability of being recovered following use of both Tet and Rappaport R-10 media than any other medium.

For the large study all GN-R cultures were incubated overnight at 37 °C, then struck onto brilliant green agar with sulfadiazine (BGS) plates. Additionally, the T48-R culture was also struck onto brilliant green agar plus novobiocin (BGN) and xylosine-lysine-tergitol-4 (XLT-4) plates. All plates were incubated overnight at 37 °C. Colonies having the typical appearance of *Salmonella* were picked to triple sugar iron and lysine iron agar slants. All slants were incubated overnight at 37 °C. Presumptive positive isolates were serogrouped using serogroup specific typing sera (Difco, Detroit, MI) then sent to the National Veterinary Services Laboratories (NVSL) for serotyping. The sample and herd prevalence rates were 6.2% (414/6,655 positive) and 38.2% (58/152 positive), respectively. Additionally, *Salmonella* spp. were recovered from 173 pens (17.5%; 173/988). The

number of serotypes recovered from the positive farms ranged from one to six (one serotype was recovered from 35 farms). The five most common serotypes recovered were *S. derby* (32.4%); *S. agona* (13.0%); *S. typhimurium* (copenhagen) (11.4%); *S. brandenburg* (7.7%); and *S. mbandaka* (7.7%). Among the positive farms (n = 58), 15.1% were positive for *S. derby* while 6.6% of the farms were positive for *S. agona*. Only one serogroup was recovered from 39 (67.2%) of the farms. The most common serogroup recovered was B (72.7%) followed by C1 (11.1%). Recovery from all other serogroups was less than 5%. The likelihood of recovering an isolate belonging to serogroup B was the greatest (81.0%) followed by C1 (17.2%), G2 (12.1%), and D1 (6.9%). Untypable, O group 16, or nonmotile isolates were recovered from 22.4% of the feedlots. *Salmonella* spp. were most often recovered from samples cultured in T48-R (88.9%). Culture in GN-R was least sensitive. Identification of positive farms was best achieved by culture in T48-R (96.6%) when compared to GN-R (3.4%). Interestingly, the 2 farms that were missed in T48-R were positive by culture in GN-R, only.

Antimicrobic susceptibility of *Salmonella* spp. — In participation with FSIS, APHIS, and FDA, baseline antimicrobic susceptibility data will be generated from the *Salmonella* isolates which have been recovered from several NAHMS and FSIS surveys. Specifically, the antimicrobic of interest is the fluoroquinolone, as it's release for use in food animals is imminent. These efforts are being coordinated for use in a national monitoring surveillance program with the FDA, CVM, APHIS, and CDC.

IMPACT/TECH TRANSFER B: This information has furthered our knowledge regarding the carrier state of *Salmonella* in swine. Results from the NAHMS Survey give a better perspective on the epidemiology of *Salmonella* in swine from a national perspective. This work has been published and presented to commodity groups and other professionals at scientific meetings.

OBJECTIVE C: Define the porcine immune response to acute and chronic *Salmonella* infection focusing on mechanisms to reduce or eliminate the pathogenic organism.

PROGRESS C: Porcine neutrophil function was characterized after *S. choleraesuis* inoculation. Using our recently developed porcine neutrophil phagocytosis assay, we confirmed an earlier observation that a twofold increase in the rate of uptake occurs early after *S. choleraesuis* infection (approximately day 2 post-exposure). This rate increase does not include a decreased rate of uptake in the first 48 hrs as previously believed. The increased rate phenomenon is not dependent on the age of the pig. We still hypothesize that this early lag period following exposure may provide an opportunity for colonization and/or replication to levels that facilitate establishment of a carrier-state or clinical infection.

In refinement of our porcine stress model, optimal doses and routes for administration of 2-deoxy-D-glucose (2DG), a metabolic stressor, were determined. Studies are now being planned to combine our stress model with our *salmonella* model to determine the effects

of stress on porcine salmonellosis. Our goal is to confirm that marketing stress does in fact affect porcine immunity and bacterial shedding at the time of slaughter.

In further development of the porcine tumor necrosis factor (TNF) assay, polyclonal antibodies against porcine TNF were developed for use in our TNF bioassays. We determined that a porcine kidney cell (PK-15) based bioassay was 15 times more sensitive than a WEHI (clone 13) bioassay for detection of porcine serum TNF.

We have successfully expressed and partially purified recombinant porcine TNF receptor 1 (rpTNFR-1). We are now attempting to produce monoclonal antibodies to this receptor.

We are in the process of evaluating possible interactions between stress, porcine reproductive and respiratory syndrome (PRRS), *Salmonella*, and potential affects on the porcine immune system. Preliminary results indicate a possible affect on B cell function.

IMPACT/TECH TRANSFER C: Control of salmonellosis in swine would increase herd health, increase profits for producers, and increase the wholesomeness of pork for the consumer. Regulation of neutrophil phagocytosis could be a useful method for the control of *Salmonell*a in swine. Knowledge gained from our stress studies also will benefit both producer and consumer since studies are aimed at reducing or eliminating the potential ill effect(s) of transportation and marketing stress on porcine immunity and subsequent *Salmonella* spp. shedding at time of slaughter. Because the PK-15-based bioassay was more sensitive for detecting porcine TNF in serum, this bioassay may be particularly useful in the study of infectious disease processes of swine. Results from our TNF receptor studies may provide important insights into methods to block endotoxemic shock in swine.

OBJECTIVE D: Identify methods to control *Salmonella* in swine.

PROGRESS D: We have initiated studies of *Salmonella* spp. in swine on the farm by following a group of 100 pigs from farrow through to finish. Pigs were tagged at one week of age, sera and rectal swabs were collected from each pig. Visits were made to the farm at 4, 9, and 14 weeks. During each visit, pigs were bled and rectal samples were collected. Additionally, 10 pigs were necropsied to determine the tissues positive for *Salmonella*. Visits will also be made at 18 weeks, and then at slaughter when 20 pigs will be necropsied on the farm and 20 pigs will be followed through to the packing plant. For each visit, samples were collected throughout the farm from all production units on the farm and rodent traps also were checked and rebaited. Through 9 weeks we have recovered *Salmonella* from rectal swabs and from other units within the farm environment, but tissues and rodents have been negative. Serology will not be completed until after slaughter. These data suggest that recovery of *Salmonella* on the farm may be variable with few long-term carriers.

The use of a mucosal competitive exclusion culture has been shown to reduce/eliminate *Salmonella* spp. in poultry. We hypothesized that a similar culture derived from a

Salmonella-free pig also would reduce/eliminate *Salmonella choleraesuis* in pigs. A mucosal competitive exclusion culture was produced from the cecum of a six-week-old pig (MCES). Pigs from 5 sows were inoculated with MCES. The culture was propagated in PRAS broth at 37 °C and 5 mL was given by oral gavage to newborn pigs within 6 hrs post-farrowing (PF) and again at 24 hr PF. All pigs were challenged with 10^3 CFU *Salmonella choleraesuis* at 48 hrs PF by intranasal instillation, including pigs from 2 sows which had not been given MCES. Clinical signs and rectal swabs were monitored daily and pigs were allowed to suckle throughout the experiment. All pigs were necropsied on D7 PF and qualitative (10 tissues) and quantitative (2 tissues) bacteriology was conducted. Clinical signs were inapparent in all pigs throughout the experiment. Recovery of *Salmonella* from rectal swabs was variable. However, 47% of the tissues were positive from the MCES treated pigs versus 90% positive tissues from the control pigs. A 2–5 $\log_{10}$ reduction of *Salmonella* in the cecal contents (CC) or ileocolic junction (ICJ) was observed in the MCES treated pigs when compared to the controls (*Salmonella* was cleared from the ICJ and CC in pigs from 2 and 1 sows, respectively). These data indicate that use of MCES reduces *S. choleraesuis* in suckling pigs and suggests that MCES may be a practical solution for control of *Salmonella*.

IMPACT/TECH TRANSFER D: This information will further our knowledge regarding control and elimination of the carrier in swine. In addition, development of new intervention strategies also may help in the reduction/elimination of the carrier animals. This work has been published in proceedings format and presented to commodity groups and professionals at scientific meetings.

PUBLICATIONS:

Baum, D. H., D. L. Harris, P. J. Fedorka-Cray, G. BeVier, R. Rankin, and B. Homann. 1996. Environmental levels of *Salmonella* in finishing buildings, pp 178–179. 1995 Swine Research Report, Iowa State University, University Extension, Ames, IA.

Baum, D. H., D. L. Harris, B. Nielsen, P. J. Fedorka-Cray, G. BeVier, B. Homann, and R. Rankin. 1996. Comparison of the Danish MIX-ELISA to culture for the detection of *Salmonella* in finishing pigs at or near slaughter, p 177. 1995 Swine Research Report, Iowa State University, University Extension, Ames, IA.

Davies, P. R., W. E. Moran Morrow, F. T. Jones, J. Deen, P. J. Fedorka-Cray, and J. T. Gray. 1996. Elevated risk of *Salmonella* shedding by market-age hogs in a barn with open flush gutters. *J. Am. Vet. Med. Assoc.* (In press).

Fedorka-Cray, P. J. 1996. The connection between *Salmonella*, swine, and food safety, pp 25–45. Proceedings of the George Young Conference, Lincoln, NE.

Fedorka-Cray, P. J., J. T. Gray, and L. A. Thomas. 1995. Comparison of culture media for the isolation of *Salmonella* species from animal species, pp 181–184. Proceedings of the Food Safety Consortium, Kansas City, MO.

Fedorka-Cray, P. J., J. T. Gray, and L. A. Thomas. 1996. Comparison of culture media for the isolation of *Salmonella* species from swine feces, pp 191–193. 1995 Swine Research Report, Iowa State University, University Extension, Ames, IA.

Gray, J. T., P. J. Fedorka-Cray, T. J. Stabel, and T. T. Kramer. 1996. Natural transmission of *Salmonella choleraesuis* in swine. *Appl. Environ. Microbiol.* **62**:141–146.

Gray, J. T., R. W. Wills, P. J. Fedorka-Cray, J. Zimmerman, and L. Hoffman. 1996. Concurrent infection of porcine reproductive and respiratory syndrome virus and *Salmonella choleraesuis* in swine, pp 585–588. Proceedings of the 27th Annual Meeting of the American Association of Swine Practitioners, Nashville, TN.

Libby, S. J. and P. J. Fedorka-Cray. 1996. Control of Salmonella virulence in the natural host. Proceedings of the 1st International Symposium: Ecology of *Salmonella* in Pork Production, Ames, IA. (In press).

McKean, J., P. Fedorka-Cray, I. Wesley, J. Dickson, P. Holden, and E. Murano. 1996. Segregated early weaning — control of food safety organisms. Proceedings of the 1st International Symposium: Ecology of *Salmonella* in Pork Production, Ames, IA. (In press).

Nisbet, D. J., D. E. Corrier, P. J. Fedorka-Cray, and J. R. DeLoach. 1996. Use of defined competitive exclusion cultures to enhance colonization resistance to enteric pathogens. Proceedings of the 1st International Symposium: Ecology of *Salmonella* in Pork Production, Ames, IA. (In press).

Smith, D. R., P. J. Fedorka-Cray, K. V. Brock, T. E. Wittum, P. S. Morley, R. Mohan, K. H. Hoblet, and L. J. Saif. 1996. An investigation of the role of bovine coronavirus, and other risk factors, in the occurrence of winter dysentery in dairy cattle. Ohio State University Animal Science Research Highlights, Ohio State University, Wooster, OH. (In press).

Stabel, T. J., P. J. Fedorka-Cray, and J. T. Gray. 1996. *Salmonella* immunity: Development of a neutrophil phagocytosis assay and stress model in swine. Proceedings of the 1st International Symposium: Ecology of *Salmonella* in Pork Production, Ames, IA. (In press).

Turney Harris, I., P. J. Fedorka-Cray, J. T. Gray, L. A. Thomas, and K. Ferris. 1996. *Salmonella* in the swine feed environment and associated herd risk factors. *J. Am. Vet. Med. Assoc.* (In press).

CONTROL OF *SALMONELLA* AND *ESCHERICHIA COLI* O157:H7 IN LIVESTOCK DURING THE PREHARVEST PERIOD

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GENERAL OBJECTIVE: Reduce the prevalence of infection with enterohemorrhagic *Escherichia coli* O157:H7 and *Salmonella* in livestock animals and meat products.

OBJECTIVE A: Develop rapid, accurate and sensitive tests for the identification of animals infected with, and products contaminated with *Escherichia coli* O157:H7.

PROGRESS A: Monoclonal antibodies (Mabs) developed against *E. coli* O157:H7 have been incorporated into standard ELISA formats and into more "user friendly" rapid formats. Both types of tests have been used to identify contaminated meat products and fecal samples. Characterization of these antibodies indicated lack of specificity in available typing reagents, especially for H7. Mapping and sequencing of H7-specific epitopes using these Mabs demonstrated that the H7 specific sequences differed only by a few amino acids from those of H23 and H24, possibly indicating a common ancestry and providing a rational basis for cross-reactivity problems with these serotypes.

IMPACT/TECH TRANSFER A: A competitive process to identify the most robust test formats for commercialization is in its final stages. Transfer of the relevant Mabs to a company for production of a rapid test for *E. coli* O157:H7 is imminent.

OBJECTIVE B: Develop rapid, accurate and sensitive tests for the identification of animals infected with, and products contaminated with *Salmonella* spp.

PROGRESS B: Validation of the use of recombinant *Salmonella* antigens to detect persistently infected carrier cattle is ongoing using several *Salmonella* serotypes obtained from field outbreaks. In addition, Mab-based detection methods for genus-, group- and serotype-specific identification of *Salmonella* infected livestock are in development. Application of these tests in field and laboratory diagnostic settings has demonstrated their efficacy in identifying *Salmonella* infections. Further development of PCR for detection of *Salmonella* nucleic acid has resulted in reduction of total test times below 15 min.

IMPACT/TECH TRANSFER B: These tests are particularly relevant to testing associated with proposed meat safety regulations. Substantial interest in these methodologies has been indicated by several major diagnostic manufacturers. Initial steps to transfer these technologies have been taken and final transfer is expected in FY-97.

PUBLICATIONS:

Kwang, J. and E. T. Littledike. 1995. Production and identification of recombinant proteins of *Salmonella typhimurium* and their use in detection of antibodies in experimentally challenged animals. *FEMS Microbiol. Lett.* **130**:25–30.

Kwang, J., E. T. Littledike, and J. Keen. 1996. Use of the polymerase chain reaction for *Salmonella* detection. *Lett. Appl. Microbiol.* **22**:46–51.

Wittum, T. E. and C. E. Dewey. 1996. Partial budget analysis of sow *Escherichia coli* vaccination. *Swine Hlth. Prod.* **4**(1):9–13.

He, Y., J. E. Keen, R. B. Westerman, E. T. Littledike, and J. Kwang. Monoclonal antibodies for detection of the H7 antigen of *Escherichia coli*. *Appl. Environ. Microbiol.* **62**(9): (In press).

Kwang, J., R. Wilson, S. Yang, and Y. He. 1996. Mapping the H7-serospecific domain of *Escherichia coli* flagellin. *Clin. Diagn. Lab. Immunol.* **3**(5): (In press).

PREVENTION OF LOSSES FROM COLIBACILLOSIS AND *ESCHERICHIA COLI* O157:H7 IN CATTLE AND SWINE

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OBJECTIVE A: Identify site and mechanism of *E. coli* O157:H7 colonization in cattle.

PROGRESS A: Epidemiologic evidence identifies cattle as important reservoirs of O157:H7 *E. coli*, a foodborne pathogen that causes hemorrhagic colitis and hemolytic uremic syndrome in humans. An important strategy for reducing the risk of O157:H7 infections in humans is to reduce the level of O157:H7 *E. coli* in cattle. This will result in decreased levels of O157:H7 in beef and dairy products. Naturally or experimentally-infected cattle shed low levels of O157:H7 *E. coli* in their feces for a long time, but we do not yet understand when, where or how cattle become infected with O157:H7 bacteria on the farm or where or how these bacteria live in the bovine intestines. Weaned calves and adult cattle remain asymptomatic after natural or experimental infection with O157:H7 *E. coli*. As newborn animals are generally very susceptible to enteric infections, we performed experimental challenge studies in newborn calves and demonstrated that newborn calves become infected and develop diarrhea. The severity of disease increased with the time post-infection, as only 2 of 7 animals developed diarrhea 18 hrs post-infection, while 3 of 3 animals developed diarrhea 3 days post-infection. The diarrhea at 3 days post-infection was severe, 2 animals required extensive veterinary care (fluid therapy) and one died. Large numbers of O157:H7 (105–108) were found in the large intestine, ileum, and feces of O157:H7-infected animals. Microscopic examination of intestinal tissues from infected calves at both 18 hrs and 3 days post-infection showed that the O157:H7 *E. coli* bacteria were attached to and damaged the intestinal surface. Immunohistochemical procedures using antibodies specific for O157:H7 confirmed that the bacteria associated with the intestinal damage were O157:H7 *E. coli*.

IMPACT/TECH TRANSFER A: The finding that O157:H7 *E. coli* bacteria are present in the small and large intestine as-well-as feces may impact upon the slaughtering of infected animals. The entire intestinal tract, not just feces, could be a possible source of O157:H7 at slaughter and result in beef contaminated with O157:H7 *E. coli* bacteria. As experimental infection of newborn calves with O157:H7 results in diarrhea, newborn calves with diarrhea on the farm are a potential source of O157:H7. Past studies have demonstrated that 1–2% of older calves (> 3 weeks) shed O157:H7, but the incidence of O157:H7 in newborn calves (< 3 weeks) with diarrhea is unknown and needs to be

determined. These newborn calves could infect producers and veterinarians directly and be a source of infection for older cattle on the farm. Prompt treatment or removal of these animals from the herd will decrease the direct risk to producers and veterinarians and reduce the prevalence of O157:H7 in the herd. This herd reduction might decrease the level of O157:H7 *E. coli* bacteria in cattle as they enter the slaughter plant and positively impact the safety of beef and dairy products.

OBJECTIVE B: Identify methods to reduce shedding of O157:H7 and other Shiga-like toxin producing *E. coli* (SLTEC) pathogenic for humans.

PROGRESS B: O157:H7 *E. coli* bacteria which are pathogenic to humans produce Shiga-like toxins, contain a large 60 megadalton plasmid and usually contain the eaeA gene which encodes intimin (see Objective C). *E. coli* of serotypes other than O157:H7 which have these virulence attributes can cause human disease and have been isolated from cattle. Therefore, therapies that reduce shedding of all SLTEC would be advantageous to the cattle industry and improve food safety. We have isolated a bacterial strain that produces microcin (a small molecule that prevents the growth of certain bacteria) from a calf experimentally infected with O157:H7. Not only did this microcin inhibit the growth of O157:H7 *in vitro*, it also inhibited the growth of a wide variety of pathogenic and nonpathogenic *E. coli in vitro*. This single animal did shed O157:H7 in its feces but at a reduced rate relative to other calves experimentally infected with O157:H7. Further *in vivo* studies are in progress to determine if this reduction was due to the presence of this microcin-producing bacteria or just by random chance.

Another promising approach to reduce the level of O157:H7 in cattle is to stimulate a protective intestinal immune response. The best method of stimulating a protective immune response is by orally vaccinating animals with live strains. However, these strains must be avirulent so that the vaccine strain itself is not a potential foodborne pathogen. We have conducted virulence testing on potential live vaccines that could be used to reduce O157:H7 levels in cattle. Some of the strains tested were extremely virulent and are not useful as vaccines in cattle. One strain that likely has reduced virulence because it is an SLTEC strain that has lost its ability to produce the Shiga-like toxin appears to have reduced virulence in calves. We have previously used a toxin-negative SLTEC strain as a vaccine for a naturally-occurring SLTEC disease of weaned pigs, edema disease. This strain was safe and prevented edema disease in swine. The SLTEC that causes edema disease in swine have a mechanism of colonization that differs from the SLTEC that are carried by cattle and also cause human disease. However, the success of the toxin-negative vaccine strain in swine supports the usefulness of a similar approach for SLTEC in cattle.

IMPACT/TECH TRANSFER B: Further testing of the microcin-producing bacterial strain and the toxin-negative O157:H7 strain will determine if they are able to reduce levels of O157:H7 and other SLTEC in cattle. Both of these strains will be easy for producers and veterinarians to use because they are orally administered. Either type of

bacteria could be given to a whole herd of cattle at one time by adding one of the strains to the feed or water. Another major advantage to these strains is that they could be administered to cattle of various ages. Therefore, the oral administration could be timed to have maximum effectiveness at critical control points in the preharvest period, such as arrival in the feedlot and prior to slaughter. These strains have the potential to decrease foodborne disease in humans caused by O157:H7 or other SLTEC by reducing their levels in cattle in the feedlot and before slaughter.

OBJECTIVE C: Determine the role of known virulence attributes in pathogenesis of O157:H7 and SLTEC infection in healthy cattle.

PROGRESS C: Determining what bacterial genes, proteins and other molecules are involved in intestinal colonization by O157:H7 and SLTEC are required for developing rational approaches to reduce colonization in cattle. Intimin is an outer membrane protein of O157:H7 and other SLTEC that is necessary for the intimate attachment of the bacteria to intestinal epithelial cells, an important factor in intestinal colonization. Others have demonstrated that when O157:H7 lose the ability to produce intimin because of mutations in the eaeA gene (the gene that encodes intimin), they cannot colonize and cause damage to the intestinal cells of newborn pigs.

We demonstrated that intimin also plays an important role in the infection of newborn calves. Calves that were infected with wild type O157:H7 *E. coli* (*eaeA*$^{+}$) developed diarrhea, had intestinal lesions, and high levels of O157:H7 *E. coli* in the intestines and feces at one or two days post-inoculation. In contrast, calves challenged with a mutant strain of O157:H7 *E. coli* (*eaeA*$^{-}$) had no diarrhea, no intestinal damage and lower levels of O157:H7 in their intestines and feces. When the ability to make intimin was restored to the *eaeA*$^{-}$ mutant, the ability to cause diarrhea, damage and grow in the intestines was restored. Studies are currently in progress to determine the role of intimin in older cattle.

IMPACT/TECH TRANSFER C: This work identified an important bacterial virulence factor that has a role in O157:H7 colonization of cattle. These data provide insight into possible therapies for preventing or reducing SLTEC infections in cattle by targeting intimin. Production of high levels of intestinal antibodies directed against intimin may reduce shedding of O157:H7 and other SLTEC in cattle. Alternatively, intimin or similar molecules could be used to competitively block the adherence of O157:H7 and other SLTEC in the intestinal tract of cattle. Both methods would reduce the level of O157:H7 and SLTEC in cattle and improve the safety of beef and dairy products.

PUBLICATIONS:

Bosworth, B. T., J. E. Samuel, H. W. Moon, A. D. O'Brien, V. M. Gordon, and S. C. Whipp. 1996. Vaccination with genetically modified Shiga-like toxin IIe prevents edema disease in swine. *Infect. Immun.* **64**:55–60.

Dean-Nystrom, E. A., D. Burkhardt, B. T. Bosworth, and M. W. Welter. 1996. Prevalence of F18ac (2134P) fimbriae on K-88 negative *Escherichia coli* isolates from weaned pigs with diarrhea. *J. Vet. Diagn. Invest.* (In press).

Johnson, R. P., W. C. Cray, Jr., and S. T. Johnson. 1996. Serum antibody responses of cattle following experimental infections with *Escherichia coli* O157:H7. *Infect. Immun.* **64**:1879–1883.

Tortorello, M. L., D. S. Stewart, and W. C. Cray, Jr. 1996. Rapid identification of *Escherichia coli* O157:H7 in bovine feces using the antibody-direct epifluorescent filter technique (AB-DEFT). *Vet. Microbiol.* (In press).

CONTROL OF *CAMPYLOBACTER JEJUNI* IN POULTRY

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OBJECTIVE A: Create, produce, and optimize the performance of a vaccine against the flagella of *Campylobacter jejuni*.

PROGRESS A: We have previously reported the creation and production of a vaccine against *Campylobacter jejuni* that was made by DNA engineering that showed almost 50 percent efficacy in initial trials. We have since discovered that the standard method for extracting the protein that was being used in production of the vaccine was causing denaturation and a loss of functionality. In the last year we have instituted new protocols for protein purification that has produced active protein. Analysis of the efficacy of the newly purified vaccine have been hampered by failures of control birds becoming infected after innoculation. A new formulation of the vaccine has been designed that should be effective against all strains of *Campylobacter*. The new formulation will be optimized and tested against strains of *Campylobacter* with proven ability to readily colonize chickens.

IMPACT/TECH TRANSFER A: A highly efficacious vaccine against *Campylobacter* in chickens will provide a safe and economical method for controlling the foodborne pathogen. Our flagellin-LTB fusion vaccine shows promise in being such an effective vaccine. An agreement has been made with Intervet, International (5830 AA Boxmeer, The Netherlands) to determine if there are common interests in developing this vaccine.

OBJECTIVE B: Identify components of *C. jejuni* that the organism requires for inhabiting the intestines of chickens and that may serve as vaccine candidates.

PROGRESS B: We previously reported finding a protein from *Campylobacter* that we designated ompH1 that has forms that correlate well with the ability of the organism to colonize chickens. Recently, we found that the gene that expresses the protein was present in every strain that was tested with only slight variability. Tests are underway to determine if intervention strategies directed against the ompH1 protein are likely to be effective in preventing colonization of chickens by *C. jejuni*.

IMPACT/TECH TRANSFER B: In general, a flagellin based vaccine does have potential for failure due to the variability among strains and the possibility that the vaccine

would not have enough identity with an infecting strain to insure protection. In order to stay away from this problem we are looking for components of *Campylobacter* that are less variable, as ompH1 appears to be, and that are still immunologically accessible, and we also are looking for integral parts necessary for colonization, which is still to be determined for ompH1.

OBJECTIVE C: Determine the degree of variability of the flagellin genes of *C. jejuni* and evaluate for effect on vaccine performance and for potential on tracking strains of *C. jejuni* in epidemiological studies.

PROGRESS C: The complete nucleotide sequences of the flagellin gene for 15 strains have now been analyzed. One major finding was of a conserved portion of the gene that led to the reformulation of the vaccine mentioned in Objective A. A short variable region also was identified that can easily be sequenced and analyzed for strain differentiation. It was found that the degree of differentiation achieved by such analysis is epidemiologically significant. Further analysis of the data has indicated that exchange of fragments of DNA between strains of *Campylobacter* may be a frequent event.

IMPACT/TECH TRANSFER C: The sequence of the flagellin gene from *Campylobacter jejuni* has proven to be highly variable. However, there is a portion that is fairly stable and has served as the model for a new formulation of a vaccine that potentially will impart immunity against all strains of *Campylobacter*. Sequence based differentiation of strains of *Campylobacter* as developed in this laboratory will be used by scientists at the Centers for Disease Control and will be applied to epidemiological studies to help determine the source of *C. jejuni* in poultry flocks. Once sources are identified, intervention strategies can be designed.

OBJECTIVE D: Improve methodology for detection and enumeration of *Campylobacter*.

PROGRESS D: Alternative gas generating systems and containers are being evaluated to obtain the optimum combination for growth and enumeration of *Campylobacter*. We also are exploring modifications of the bioMerieux Vidas system as a rapid method for *Campylobacter* detection.

IMPACT/TECH TRANSFER D: These efforts may lead to simplified and improved detection methods for *Campylobacter* spp. in food microbiology laboratories both in public and private sectors. We anticipate extending our CRADA with bioMerieux (St. Louis, MO) to continue studies to automate the detection of the organism. As we are able to simplify the technology for cultural detection of *Campylobacter* spp., the FSIS may be able to incorporate this technology in their laboratories.

OBJECTIVE E: Determine whether yeast may be administered to poultry to reduce *Campylobacter* colonization.

PROGRESS E: We have been evaluating the yeast, *Saccharomyces boulardii*, for ability to reduce colonization of broilers with *Campylobacter* spp. Previous experiments in our lab have demonstrated that *S. boulardii* can diminish colonization of chicks with *Campylobacter* when the yeast is administered by oral gavage. Other more commercially applicable methods to administer yeast to poultry are being analyzed. The viable yeast has been successfully incorporated onto pelleted feed and we have conducted several feeding trials to determine appropriate dose levels and times of administration for reducing pathogen colonization. Feeding the yeast throughout the grow-out period at a low level has not been observed to reduce *Campylobacter* colonization. However, feeding the yeast at an elevated level for a brief period prior to feed withdrawal and transport of the birds to the processing facility was successful in preventing the typical increase in *Campylobacter* populations associated with the stresses of transport. We are currently conducting trials to determine how combinations of various yeast treatments and other intervention methods such as Mucosal Competitive Exclusion cultures may interact to further reduce *Campylobacter* colonization of poultry.

IMPACT/TECH TRANSFER E: The ability of *Saccharomyces boulardii* to prevent increases in *Campylobacter* colonization during transport of poultry has great potential as there is currently no other reported approach with this capability. The technology is being supported through a CRADA with Lallemand, Inc. (Montreal, Canada), a patent has been applied for, and the technology has been licensed by Lallemand.

OBJECTIVE F: Determine whether a relation exists between levels of *Campylobacter* spp. found on the farm with levels found on processed poultry carcasses from that flock.

PROGRESS F: Twenty flocks (20,000 broilers in each flock) were sampled to assess the relation indicated in the Objective. One day prior to slaughter, 50 fecal samples per flock were obtained and enumerated for the organism. The next morning these were the first flocks to be processed. Fifty carcass rinses were taken from the post-chill processing location. Levels from the droppings ranged from non-detectable to about 10^8 CFU/g, and carcass levels ranged from non-detectable to about 10^6 CFU/carcass. Levels in the fecal samples were not correlated ($r = 0.3382$) with the levels found on completely processed carcasses. The mean level of *Campylobacter* spp. in the fecal samples was $10^{5.2 \pm 1.3}$ CFU/g. We determined that only 24 randomly distributed droppings would be adequate to predict the level *Campylobacter* spp. in a flock of birds at a 95% confidence level. The mean level of *Campylobacter* spp. on the samples was $10^{2.9 \pm 0.8}$ CFU/carcass. We determined that only 9 random samples would predict the level of *Campylobacter* spp. found on chilled carcasses from among a flock of processed birds at a 95% confidence level. The highest levels found on the carcasses occurred when the greatest rainfall (1.96 inches) fell on the day of transport and processing. The next highest level on the carcasses among the 20 processed flocks also was associated with rainy weather (1.05 inches). We

observed that a 25-fold difference in carcass levels of *Campylobacter* spp. existed. Determining the reasons for these differences should lead to a reduced incidence of carcasses with high levels of *Campylobacter* so that consumer exposure can be reduced. We did not observe increased numbers of *Campylobacter* on the carcasses throughout the processing of the flocks. Therefore, processing does not appear to contribute to an increase in the numbers of *Campylobacter* on the processed carcasses.

IMPACT/TECH TRANSFER F: Special efforts must be directed toward reducing the devastating effect that feed withdrawal and transport have on the high levels of *Campylobacter* on the fully processed carcasses. These might include application of yeast (described above) in finishing feed, the low technology option of covering the transport vehicles to diminish exposure of the chickens to rainy weather, and the insights obtained from determining the reasons for the 25-fold differences in *Campylobacter* levels also may be applied to reducing the levels of this organism.

PUBLICATIONS:

Line, E. 1996. Potential applications of Saccharomyces cerevisiae to limit pathogen colonization in boiler chickens, p 24. Center for Food Safety and Quality Enhancement Proceedings, Atlanta, GA.

Zhao, S., J. Meng, M.P. Doyle, R. Meinersmann, G. Wang, and P. Zhao. 1996. A low molecular weight outer-membrane protein of *Escherichia coli* O157:H7 associated with adherence in INT407 cells and chicken caeca. *J. Med. Microbiol.* **45**:90–96.

PREVENTION IN LIVESTOCK OF POTENTIAL HUMAN FOODBORNE PATHOGENS

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OBJECTIVE A: Develop rapid and sensitive techniques to detect and quantify *Campylobacter*, *Listeria*, and other emerging foodborne bacterial pathogens in livestock.

PROGRESS A: *Campylobacter jejuni* and *C. coli* are the major causes of human foodborne illness. We designed a multiplex PCR to distinguish *C. jejuni* from *C. coli*, using primers targeting the *fla*A gene and nucleotide sequences unique to *C. jejuni*. Initial studies demonstrated that *C. jejuni* yielded two PCR products (450 bp and 160 bp) while *C. coli* generated a single product (450 bp). We compared conventional biochemical testing with PCR to differentiate *C. jejuni* from *C. coli*. Of 85 field strains examined, PCR and conventional testing agreed for 82 strains. PCR identified three isolates which could not be identified conventionally because of their atypical biochemical patterns.

Arcobacter spp. are aerotolerant *Campylobacter*-like organisms which have been recovered from livestock, meats, and have been associated with human enteritis. Of the 4 species of *Arcobacter*, *A. butzleri* is regarded as the human pathogen. A PCR assay, targeting the 16S rRNA gene of *Arcobacter* spp. was designed. The PCR product (1,223 bp) was obtained only with strains of *Arcobacter butzleri*, *A. cryaerophilus*, and *A. skirrowii*. No amplification occurred with species of *Campylobacter* or *Helicobacter*. The PCR assay was utilized to detect *Arcobacter* from P-80 enrichment media. Of 207 swine fecal samples, 69% (142/207) were positive by darkfield and culture, whereas 68% (141/207) were positive by PCR. In one case (0.5%) a confirmed culture-positive sample was negative by PCR. These data indicated the efficiency of PCR, which bypassed subjective darkfield microscopy and the expense of media required for subculture.

Listeria monocytogenes is a human foodborne pathogen with an estimated mortality rate of 35%. We developed a multiplex PCR to distinguish *L. monocytogenes* from other *Listeria* species. PCR primers targeted the 16S rRNA gene of the genus *Listeria* and the listeriolysin O (LLO) gene unique to *L. monocytogenes*. In this assay, *L. monocytogenes* yielded two PCR products (938 bp and 174 bp), whereas a single PCR product (938 bp) was seen in other *Listeria* species. Specificity of the multiplex PCR assay was evaluated using field isolates of *Listeria* (n = 96) which previously were identified by biochemical methods and/or by their reactivity with the LLO probe. The PCR assay correctly distinguished *L. monocytogenes* (n = 79 isolates) from *L. innocua* (n = 8), *L. welshimerii*

(n = 2), *L. grayii* (n = 2), and *L. seeligeri*.(n = 2). It failed to amplify field isolates of *Erysipelothrix* (n = 7), which is closely related phylogenetically to *Listeria* spp.

IMPACT/TECH TRANSFER A: These results indicate the suitability of PCR-based methods to rapidly identify potential human foodborne pathogens. These PCR assays are highly reproducible, provides definitive identification in a shorter time than conventional biochemical testing, and can be applied to screening large numbers of samples encountered in routine surveys. The assays are technically "user friendly", simple to perform, but requires a thermal cycler, which can be accommodated in routine microbiological laboratories.

OBJECTIVE B: Define the epidemiological role of swine and cattle as carriers of *Campylobacter*, *Listeria*, and other emerging foodborne bacterial pathogens.

PROGRESS B: We utilized multiplex PCR to estimate the prevalence of *C. jejuni* and *C. coli* in healthy livestock. Livestock feces was streaked onto modified charcoal-cefazolin-sodium deoxycholate agar (mCCDA) supplemented with 0.01% amphotericin B. After incubation (48 hrs in an atmosphere of 5% O_2, 10% CO_2, 85% N_2), bacterial colonies were processed directly for PCR. Of 1,051 pig fecal samples examined, 69% were positive for *C. coli* and 3% were positive for *C. jejuni*. Of 2,085 cattle fecal samples processed, 37.7% were positive for *C. jejuni* and 2% were positive for *C. coli*.

Because the *Arcobacter* PCR assay was comparable to identification by darkfield microscopy and subculture to blood agar, the PCR assay was used to estimate the prevalence of *Arcobacter* in healthy livestock. *Arcobacter* spp. were detected in 42% of swine (n = 1,002) and in 14.3% of dairy cattle (n = 1,682) fecal samples. In a pilot study of 100 mechanically deboned turkey products, 91% of the enrichment samples were positive for *Arcobacter* by PCR. When the PCR products were Southern blotted and hybridized with the *A. butzleri*-specific probe, 87.9% of the 100 samples were positive for *A. butzleri*.

IMPACT/TECH TRANSFER B: These results indicate the suitability of PCR-based methods in large scale testing of livestock and foods to detect potential human foodborne pathogens.

OBJECTIVE C: Evaluate management practices to reduce foodborne bacterial pathogens in swine.

PROGRESS C: Segregated early weaning (SEW) is a production technology to reduce or to eliminate transmission of common bacterial and viral swine pathogens. In order to determine if SEW also could reduce potential zoonotic foodborne bacteria, we compared the prevalence of *Campylobacter* spp. and *Arcobacter* spp. in SEW and in conventionally reared market hogs. Pigs were screened as they entered the SEW nursery (Initial), upon

exiting the nursery prior to transfer to the distant finishing site (At Transfer), and at market weight (Market Weight). Controls were sampled at market weight while on the original premise. We reported previously that for SEW-reared animals at market weight no differences were seen in *Campylobacter* between the on-farm controls (41%) and SEW (38%) hogs. In contrast, at market weight the prevalence of *Arcobacter* spp. was higher in SEW (84%) than in control (24%) farms. A replicate trial involved 21 farms (16 pigs per farm). In this second trial as in the first, SEW piglets entered the off-site nursery at approximately 4 weeks of age and were transported to a distant finishing site at approximately 12 weeks of age. However, in contrast to the initial trial, control piglets (n = 8 per farm) did not remain on the individual control premises, but were transported to the finishing site at approximately 12 weeks of age. As summarized in Table 1, at the initial sampling upon entry into the nursery, *Campylobacter* was cultured from at least one animal from all of the farms; *Arcobacter* was cultured from pigs on 33% (7/21) of the farms. For SEW pigs at transfer, *Campylobacter* (95.4%) and *Arcobacter* (95%) were cultured from at least one animal from most of the 22 farms. For control farms at transfer, *Campylobacter* was cultured from 86.4% (19 of 22) of the premises while *Arcobacter* was detected on 9% (2 of 22) of the control sites. In Trial 2, control animals were moved to the finishing site 8 weeks prior to slaughter, in contrast to Trial 1 in which control animals remained on the control site. The consequences of transporting the control animals to within 50 yards of the SEW group at the finishing site are summarized in Table 1. Despite the low recovery of *Arcobacter* in the control animals initially and at transfer, no differences were seen in the SEW (100%) and control (86%) market weight hogs.

Table 1. Distribution of *Campylobacter* and *Arcobacter* spp. in pigs raised under SEW management at three sampling times. Data are reported as number of positive farms.

	INITIAL	AT TRANSFER	MARKET WT
SEW farms (n = 21)			
Campylobacter	21/21	21/22	12/22
Arcobacter	7/21	22/22	22/22
Control farms (n = 21)			
Campylobacter	21/21	19/22	15/22
Arcobacter	7/21	2/22	19/22

IMPACT/TECH TRANSFER C: This study was part of the first evaluation of SEW techniques to control potential zoonotic foodborne pathogens under commercial conditions. It indicates that a reduction in one potential pathogen may not ensure a reduction in other bacterial pathogens. It suggests that the benefits of one production system may be neutralized by premature movement of animal prior to slaughter.

PUBLICATIONS:

Baetz, A. L. and I. V. Wesley. 1995. Detection of anti-listeriolysin O in dairy cattle experimentally infected with *Listeria monocytogenes*. *J. Vet. Diagn. Invest.* 7:82–86.

Baetz, A. L., I. V. Wesley, and M. Stevens. 1996. The use of listerolysin O in an ELISA, a skin test, and a lymphocyte blastogenesis assay on sheep experimentally infected with *Listeria monocytogenes*, *Listeria ivanovii* or *Listeria innocua*. *Vet. Microbiol.* **51**:151–159.

Collins, C. I., I. V. Wesley, and E. A. Murano. 1996. Detection of *Arcobacter* spp. in ground pork by modified plating methods. *J. Food Prot.* **59**:448–452.

Dickson, J. S., T. R. Manke, I. V. Wesley, and A. L. Baetz. 1996. Biphasic culture of *Arcobacter* spp. *Lett. Appl. Microbiol.* **22**:195–198.

Harmon, K. M. and I. V. Wesley. 1996. Identification of *Arcobacter* isolates by PCR, pp 209–211. 1995 Swine Research Report, Iowa State University, University Extension, Kansas City, MO.

Johnson, G. C., C. W. Maddox, W. H. Fales, W. A. Wolff, R. F. Randle, J. A. Ramos, H. Schwartz, K. M. Heise, A. L. Baetz, I. V. Wesley, and D. E. Wagner. 1996. Epidemiologic evaluation or encephalitic listeriosis in goats. *J. Am. Vet. Med. Assoc.* **208**:1695–1699.

Schroeder-Tucker, L., I. V. Wesley, J. A. Kiehlbauch, G. A. Erickson, L. A. Thomas, and D. J. Larson. 1996. Phenotypic and ribosomal RNA characterization of *Arcobacter* species isolated from porcine aborted fetuses. *J. Vet. Diagn. Invest.* **8**:186–195.

Wesley, I.V., A. L. Baetz, and D. J. Larson. 1996. Infection of Cesarean-derived colostrum-deprived one-day-old piglets with *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. *Infect. Immun.* **64**:2295–2299.

Wesley, I. V., A. L. Baetz, and D. J. Larson. 1996. Infection of Cesarean-derived colostrum-deprived (CDCD) one-day-old piglets with *Arcobacter butlzeri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*, pp 204–208. 1995 Swine Research Report, Iowa State University, University Extension, Kansas City, MO.

DIAGNOSIS AND EPIDEMIOLOGY OF BOVINE TUBERCULOSIS

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OBJECTIVE A: Determine effectiveness of slaughter surveillance for detection of bovine tuberculosis.

PROGRESS A: The primary method for detecting cattle with tuberculosis in the United States is inspection of carcasses when cattle are slaughtered. Detection of tuberculosis by slaughter surveillance requires that infected cattle have visible lesions characteristic of tuberculosis at sites that are routinely inspected. We determined that not all cattle with tuberculosis have evidence of disease that would be detected when carcasses are inspected. Bovine tuberculosis was confirmed by laboratory examination in 15 cattle originating from an infected dairy herd. Four of the 15 infected cattle (26.7%) did not have visible lesions of tuberculosis in sites that are inspected during routine slaughter surveillance. These findings indicate that not all cattle with tuberculosis can be detected using only slaughter surveillance. Other methods need to be used in conjunction with meat inspection to identify all cattle with tuberculosis.

IMPACT/TECH TRANSFER A: The program for eradication of bovine tuberculosis from the United States relies on slaughter surveillance as the primary tool for detection of cattle with tuberculosis. Because not all cattle with tuberculosis have visible lesions in sites that are inspected, bovine tuberculosis cannot be eliminated from the United States unless additional methods are used to detect all infected cattle.

OBJECTIVE B: Develop improved tests for diagnosis of bovine tuberculosis.

PROGRESS B: Definitive diagnosis of bovine tuberculosis requires isolation of *Mycobacterium bovis* from tissue specimens collected at slaughter. Isolation and identification of the organism requires 8 to 12 weeks before results are available. A presumptive diagnosis can be made when lesions characteristic of tuberculosis with acid fast organisms are observed by histopathologic examination. However, it was not possible to identify the organism using previous procedures. We developed a rapid method using the polymerase chain reaction (PCR) to identify *M. tuberculosis* complex organisms, which includes *M. bovis*, in formalin-fixed paraffin-embedded tissue specimens. Results of the new test are available within one day of sample processing. Although this test does

not eliminate the need to isolate *M. bovis* from tissue specimens, it can be used as an additional tool for the National Bovine Tuberculosis Eradication Program.

IMPACT/TECH TRANSFER B: Regulatory veterinarians have requested the use of the new test for rapid diagnosis of several cases of tuberculosis. It has been especially useful for cases where isolation of *M. bovis* was not possible.

OBJECTIVE C: Determine risk of infection with *Mycobacterium bovis* from ingestion of contaminated meat products.

PROGRESS C: We used swine as an animal model to study the risk of humans becoming infected with *M. bovis* by ingestion of contaminated meat products. Swine have been used as an animal model to study a variety of human diseases. Results of our previous research demonstrated that swine are susceptible to *M. bovis* when challenged by the intratracheal, intratonsilar, intravenous, intragastric, and oral routes of inoculation. We determined that swine become infected when they are fed uncooked ground beef that contains between 10^3 and 10^6 *M. bovis* organisms. We are continuing research to determine the minimum number of organisms needed to infect swine fed *M. bovis* in uncooked meat products.

IMPACT/TECH TRANSFER C: At the conclusion of these experiments, we will be able to estimate the risk of humans becoming infected with *M. bovis* by ingestion of contaminated meat products that are not cooked.

PUBLICATIONS:

Whipple D. L., C. A. Bolin, and J. M. Miller. 1996. Distribution of lesions in cattle infected with *Mycobacterium bovis*. *J. Vet. Diagn. Invest.* **8**:351–354.

DISEASE RELATED PROBLEMS OF POULTRY PRODUCTION AND PROCESSING (OSTEOMYELITIS IN TURKEYS, PROVENTRICULITIS IN BROILERS, AND INTESTINAL STRENGTH)

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OBJECTIVE A: Determine the etiology of turkey green-liver/osteomyelitis complex (TOC), evaluate the involvement of the immune system in TOC, and develop methods to reduce the impact of TOC on turkey production.

PROGRESS A: Research on TOC is focused on determining why a small percentage of healthy-appearing processed turkeys are infected with a variety of opportunistic bacteria, affecting bone, muscle, and synovial tissue. Previous studies conducted by this group suggest that immunological dysfunction contributes to the onset of TOC. We have recently succeeded in developing a model for reproduction of these lesions using air sac inoculation of *Escherichia coli* at 5 weeks of age. When 100 bacterial cells were injected into the sir sac, TOC lesions were found in 7.4% of turkeys by 2 weeks post-inoculation. Intramuscular injection of dexamethasone, a corticosteroid known to suppress immune function, increased TOC incidence to 27% using the same number of bacteria.

IMPACT/TECH TRANSFER A: Our ability to experimentally reproduce the lesions of TOC is important for four reasons. First, field incidence of TOC is only 0.5%, a level too low for statistical evaluation of the effect of remedial measures such as antibiotic treatment or immunomodulation. This model should enable us to evaluate such measures. Second, this model is the first to demonstrate the possibility of a respiratory origin for the bacteria that cause TOC. Third, these data suggest that TOC might result from immune dysfunction and thus might be prevented by immunomodulation, and fourth, since *E. coli* air sacculitis/septicemia is considered by some to be the most important turkey disease, this model may have wide impact on turkey health. This research could lead to the reduction of TOC in turkeys, which would consequently reduce the need for the FSIS inspection procedures to identify affected carcasses.

OBJECTIVE B: Isolate and characterize the etiological agent of proventriculitis in broilers.

PROGRESS B: Proventriculitis is a problem of food safety significance because rupture of the proventriculus during processing causes carcass contamination with intestinal contents. We have established that a filtrate of affected proventricular material which is free of bacteria will cause proventriculitis when fed to day-old broilers. We also have shown that copper sulfate added to feed can cause proventriculitis and can interact with the infectious homogenate to decrease body weights and feed conversion.

IMPACT/TECH TRANSFER B: We are currently attempting to isolate a causative virus from proventricular homogenates. This may enable the development of a vaccine to prevent the condition.

OBJECTIVE C: Develop methods to increase the intestinal strength of poultry.

PROGRESS C: Intestines can become weakened and easy to tear due to the effects of disease, mycotoxins, and diet. Mechanical evisceration can then result in torn intestines which contaminate carcasses and increase the spread of potential pathogens throughout the processing plant. We have developed a sensitive method for accurately measuring intestinal strength not only to document the effects of agents which decrease intestinal strength, but also to evaluate ways to increase intestinal strength.

IMPACT/TECH TRANSFER C: The methodology developed for measuring intestinal strength is a valuable tool for research into intestinal disease. Development of methods to increase the intestinal strength would decrease the cost of poultry processing and increase product safety.

PUBLICATIONS:

Bayyari, G. R., W. E. Huff, J. M. Balog, and N. C. Rath. 1995. Experimental reproduction of proventriculitis using homogenates of proventricular tissue. *Poult. Sci.* **74**:1799–1809.

Bayyari, G. R., W. E. Huff, J. M. Balog, and N. C. Rath. 1996. Bacterial challenge of turkeys with different responses to toe-web inoculation with phytohemagglutinin-P, p 435. Proceedings of the Forty-Fifth Western Poultry Disease Conference, Cancun, Mexico.

Bayyari, G. R., W. E. Huff, J. N. Beasley, J. M. Balog, and N. C. Rath. 1995. The effect of dietary copper sulfate on infectious proventriculitis. *Poult. Sci.* **74**:1961–1969.

Huff, W. E., J. M. Balog, G. R. Bayyari, and N. C. Rath. 1994. The effect of mycocurb, propionic acid, and calcium proprionate on the intestinal strength of broiler chickens. *Poult. Sci.* **73**:1352–1356.

Huff, W. E., G. R. Bayyari, N. C. Rath, and J. M. Balog. 1996. Effect of feed and water withdrawal on green liver discoloration in turkeys. *Poult. Sci.* 75:59–61.

Wilson, M. A., L. A. Newberry, J. K. Skeeles, J. N. Beasley, G. R. Bayyari, W. E. Huff, R. W. McNew, G. G. Hein, C. E. Whitfill, and E. Haddad. 1996. Infectious bursal disease virus induced proventriculitis in maternally immune broiler chickens, p 193. Proceedings of the Forty-Fifth Western Poultry Disease Conference, Cancun, Mexico.

STRATEGIES TO CONTROL SWINE PARASITES AFFECTING FOOD SAFETY

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OBJECTIVE A: Reduce transmission of foodborne pathogens of swine by defining cytokine-regulated immune mechanisms that protect pigs against parasites that threaten food safety.

PROGRESS A: Infectivity was determined for low doses of *Toxoplasma gondii* in swine to assess risk factors for transmission with the result that only one oocyst can cause a *T. gondii* infection in pigs. Vaccination studies were initiated in pigs using irradiated *T. gondii* oocysts and showed positive antibody production; protection studies are underway. Showed the reactivity of a panel of monoclonal antibodies against porcine immune cells during the 2nd International Swine CD Workshop. RT-PCR was used to quantify interleukin-10 (IL-10) and interleukin-12 (IL-12) gene expression in pigs showing increases during infection with *Trichuris suis* concomitant with *Campylobacter* spp. Pigs inoculated with *Trichuris suis* eggs had a 10-fold increase in IL-10 expression at 40 days after inoculation compared to uninfected controls and relative to expression of the housekeeping gene, HPRT. IL-12 levels also increased, but only in pigs exposed naturally to *T. suis* on dirt and infected concomitantly with *Campylobacter* spp. These results implicate the involvement of type 2 and type 1 immune responses, respectively, during infections of pigs with worms and secondary bacterial infections.

The severe pathology seen in the colon of growing pigs associated with *T. suis* and *Campylobacter* spp. infections could be completely reversed by therapeutic drug treatment. Pigs infected for 35 days with *T. suis* were given fenbendazole therapeutically and intestinal pathology was compared to infected controls 7 days later. Worms were absent and pathology was normal following drug treatment; a similar pattern of protection and amelioration of intestinal pathology was observed with pigs immunized against *T. suis* using culture-derived parasite secreted-antigens. A model of interaction between *T. suis* and *Camplyobacter jejuni* in gnotobiotic pigs was developed. A combined *T. suis* and *C. jejuni* infection resulted in enhanced clinical disease and intestinal pathology compared to either infection given alone. This model will be used to examine the immunomodulatory events that predispose young pigs to secondary bacterial-induced enteritis.

IMPACT/TECH TRANSFER A: The effectiveness of an irradiated oocyst vaccine against *T. gondii* will provide basic information for the development of candidate antigen vaccines. When developed, these vaccines will help prevent toxoplasmosis in those herds where management control efforts cannot be completely effective, and thus increase the safety of pork products. Observations that therapeutic control of *T. suis* can ameliorate secondary bacterial infection can provide a new tool for producers to reduce the impact of infectious disease in their herds. Thus, prevention of *T. suis* infections should help decrease the incidence of foodborne bacterial infections as well.

OBJECTIVE B: Reduce transmission of foodborne pathogens of swine by identifying DNA sequences for diagnosis and for expression of specific and shared antigens for a broad spectrum anti-worm vaccine.

PROGRESS B: DNA probes were developed that differentiate *Trichinella pseudospiralis* species that are more infectious for humans. Enzymatic amplification of the expansion segment 5 sequences within domain IV of the large subunit ribosomal DNA generated distinct results among geographical isolates of *Trichinella pseudospiralis* from both avian and mammalian hosts. These markers, combined with infectivity studies, could be used to identify isolates with zoonotic consequences for humans.

A thiol and metalloprotease was characterized and cloned from *T. suis* which, in native form, are immunoprotective in pigs against *T. suis* whipworm infection. Pigs immunized twice with parasite-derived antigens in alum gave 80% protection against a challenge infection and reduced pathology related to both worm and secondary bacterial-induced lesions in the colon. *Taenia crassiceps* peptides were cloned and expressed that induce protection against the *T. crassiceps* in rodents. Mice immunized with TCA2-MBP, a fusion protein from *T. crassiceps* larval cysts, showed a 93% decrease in the number of cysts recovered after a challenge infection compared to controls. This supports the use of the recombinant peptide as a protective immunogen in large animals. mRNA from intestinal cells of *Ascaris suum* was used to construct a cDNA library that is being screened for gastrointestinal (GI) nematode common and cryptic antigens.

IMPACT/TECH TRANSFER B: Diagnostic probes are available to identify zoonotic strains of *T. pseudospiralis*. Cloned genes from *T. suis*, *A. suum*, and *T. crassiceps* are in GENBANK and represent starting reagents to develop a pan-specific GI nematode vaccine for use in swine.

PUBLICATIONS:

Arriaga, C., L. Yepez-Mulia, N. Viveros, A. Adame, D. Zarlenga, J. Lintenfels, E. Benitez, and M. Ortega-Pierres. 1995. Detection of *Trichinella spiralis* muscle larvae in naturally-infected horses. *J. Parasitol.* **81**:781–783.

Brim, T., J. VanCott, J. Lunney, and L. Saif. 1995. Cellular immune responses of pigs after primary inoculation with porcine respiratory coronavirus or transmissible gastroenteritis virus and challenge with porcine transmissible gastroenteritis virus. *Vet. Immunol. Immunopathol.* **48**:35–54.

Dubey, J., J. Lunney, S. Shen, O. Kwok, D. Ashford, and P. Thulliez. 1996. Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs. *J. Parasitol.* **82**:438–443.

Lindsay, D., D. Zarlenga, H. Gamble, F. Al-Yaman, P. Smith, and B. Blagburn. 1995. Isolation and characterization of *Trichinella pseudospiralis*, Garkavi, 1972, from a black vulture (*Coragyps atratus*). *J. Parasitol.* **81**:920–923.

Lunney, J., A. Saalmueller, P. Thomas, P. Boyd, S. Hyatt, D. Strom, S. Martin, and F., Zuckermann. 1996. Cellular immune responses controlling infectious diseases, p 307–317. *In* Swine in Biomedical Research, L. C. Schook and M. Tumbleson (eds), Plenum Publishing Corp., New York, NY.

Mansfield, L. and J. Urban. 1996. The pathogenesis of necrotic proliferative colitis in swine is linked to whipworm induced suppression of mucosal immunity to resident bacteria. *Vet. Immunol. Immunopathol.* **50**:1–17.

Urban, J., R. Fayer, S. Chen, W. Gause, M. Gately, and F. Finkelman. 1996. IL-12 protects immunocompetent and immunodeficient neonatal mice against infection with *Cryptosporidium parvum*. *J. Immunol.* **156**:263–268.

Zarlenga, D., R. Aschenbrenner, and J. Lichtenfels. 1996. Variations in microsatellite sequences provide evidence for population differences and multiple ribosomal gene repeats within *Trichinella pseudospiralis*. *J. Parasitol.* **82**:534–538.

PREVENTION & THERAPY FOR PROTOZOAN PARASITES AFFECTING FOOD ANIMALS, FOOD SAFETY, PUBLIC HEALTH

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OBJECTIVE A: Prevent illness in food animals, control food contamination and protect public health from protozoan parasites. *Cryptosporidium parvum* affecting all mammals will be a target of immunity based strategies.

PROGRESS A: Exposure of *Cryptosporidium parvum* oocysts to saturated atmospheres of several low molecular weight gases (e.g. ammonia, ethylene oxide, methyl bromide, carbon monoxide, and formaldehyde) were tested to identify potential disinfectants. Results demonstrated that ammonia, ethylene oxide and methyl bromide, but not carbon monoxide or formaldehyde would render *C. parvum* oocysts noninfectious.

Preliminary experiments using the polymerase chain reaction (PCR) technique and parasite specific primers indicate that a rapid and sensitive method for measuring infection levels of cryptosporidial stages in intestine of neonatal mice and in other infected hosts could be developed.

We assessed the immune response and composition of different immune effector cells in intraepithelial, lamina propria and draining lymph nodes of 9-day old calves infected with *Cryptosporidium parvum*. The infection elevated the numbers of T cells in these tissues implying that if immune cell migration to these tissues could be stimulated early in life, this infection could possibly be prevented in young calves.

Ducks were infected with *C. parvum* oocysts, recovered from cattle, to determine whether they could spread the parasite in their feces. It was demonstrated that these ducks could indeed release infectious *C. parvum* oocysts in their fecal material two to seven days after ingesting the parasite. Thus, migratory birds may be a factor in the wide dissemination of this parasite in the environment.

IMPACT/TECH TRANSFER A: We verified that low molecular weight gases may be useful for disinfecting instruments and small areas to eliminate infective cryptosporidial organisms. A new assay may make it possible to assess low level infections with *C. parvum* and thus test vaccines at *Cryptosporidium* challenge doses that are relevant to

human infection, and also test anti-cryptosporidial therapies in mice. We discovered a possible reason why young calves are susceptible to *C. parvum* infection and that new understanding prompted studies to accelerate maturation of the immune system as a means of preventing this infection in dairy cattle. Migratory birds are capable of disseminating infectious *C. parvum* oocysts far from the point of origin, and thus may be a factor in environmental contamination with this parasite.

PUBLICATIONS:

Elsasser, T. H., N. C. Steele, and R. Fayer. 1995. Cytokines, stress and growth modulation, pp 261–290. *In* Cytokines in Animal Health and Disease, M. Myers, and M. Murtaugh (eds), Marcel Deker, New York, NY.

Fayer, R. 1995. Effect of sodium hypochlorite exposure on infectivity of *Cryptosporidium parvum* oocysts for neonatal BALB/c mice. *Appl. Environ. Microbiol.* **61**:844–846.

Fayer, R. and R. Fetterer. 1995. Activity of benzimidazoles against cryptosporidiosis in neonatal BALB/c mice. *J. Parasitol.* **81**:794–795.

Fayer, R., T. K. Graczyk, and M. R. Cranfield. 1995. Multiple heterogenous isolates of *Cryptosporidium serpentis* from captive snakes are not transmissible to neonatal BALB/c mice (*Mus musculus*). *J. Parasitol.* **81**:482–484.

Fayer, R. and T. Nerad. 1996. Effects of low temperatures on viability of *Cryptosporidium parvum* oocysts. *Appl. Environ. Microbiol.* **62**:1431–1433.

Graczyk, T. K., M. R. Cranfield, and R. Fayer. 1995. A comparative assessment of direct fluorescence antibody, modified acid-fast-stain, and sucrose flotation techniques for detection of *Cryptosporidium serpentis* oocysts in snake fecal specimens. *J. Zoo Wildl. Med.* **26**:396–402.

Graczyk, T. K., M. R. Cranfield, and R. Fayer. 1996. Evaluation of commercial enzyme immunoassay (EIA) and immunofluorescent antibody (IFA) test kits for detection of *Cryptosporidium* oocysts of species other than *Cryptosporidium parvum*. *Am. J. Trop. Med. Hyg.* **54**:274–279.

Jenkins, M. C. and R. Fayer. 1995. Cloning and expression of cDNA encoding an antigenic *Cryptosporidium parvum* protein. *Mol. Biochem. Parasitol.* **71**:149–152.

Jenkins, M., D. Kerr, R. Fayer, and R. Wall. 1995. Serum and colostrum antibody responses induced by jet-injection of sheep with DNA encoding a *Cryptosporidium parvum* antigen. *Vaccine* **13**:1658–1664.

Urban, J. F., R. Fayer, S. J. Chen, W. C. Gause, M. K. Gately, and F. D. Finkelman. 1996. IL-12 protects immunocompetent and immunodeficient neonatal mice against infection with *Cryptosporidium parvum*. *J. Immunol.* **156**:263–268.

IDENTIFICATION AND MAPPING OF GENES INVOLVED IN PARASITIC DISEASE RESISTANCE/SUSCEPTIBILITY

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OBJECTIVE A: Identify and breed livestock and poultry that are genetically resistant to parasite infections.

PROGRESS A: Genetically defined minipigs, specifically those with swine leukocyte antigens of "a" haplotype were tested for factors that enable *Trichinella spiralis* infected pigs to react against the encysted stage of this parasite. Comparative analyses of cell subsets in local lymphoid tissues (mesenteric and submandibular lymph nodes) did not reveal any significant differences. Cytokine analyses will now be pursued.

Studies were initiated to determine whether *Toxoplasma gondii* resistant pigs can be identified and to define the genes and immune factors that encode such parasite resistance. Repeat experiments testing defined infections with low numbers of *T. gondii* oocysts were performed with 3–4 groups of 3 genetically defined miniature pigs. Results indicated that genetically, resistant pigs could be identified, especially if low doses of *T. gondii* were used for the innocula. Tissue samples have been saved so that immune mechanisms controlling this resistance can be identified; cytokine assays are underway. Once data has been collected on enough individuals, mapping studies will be pursued.

IMPACT/TECH TRANSFER A: These studies should help breeders to reduce costs of drug and vaccine treatments by selecting for parasite resistant stock. In areas where these parasitic diseases cannot be eliminated, this alternate approach should result in healthier pigs and should help prevent parasite contamination of pork products.

PUBLICATIONS:

Burgarski, D., K. Cuperlovic, and J. K. Lunney. 1996. MHC (SLA) class I antigen phenotype and resistance to *T. spiralis* infection in swine: A potential relationship. *Acta Vet. Belgrade.* **46**:115–126.

Lunney, J. K., D. R. Grimm, R. Holley-Shanks, T. Goldman, F. A. Ponce De Leon, S. Ambady, J. Pobl, J. Mendiola, L. Buoen, and C. Louis. 1996. Chromosome specific libraries and their use in genome mapping, pp 61–73. *In* Biotechnology's Role in the Genetic Improvement of Farm Animals, R. H. Miller, V. G. Pursel, and H. D. Norman (eds), American Society of Animal Science Press, Savoy, IL.

Lunney, J. K., and D. H. Sachs. 1995. The Swine Leukocyte Antigen (SLA) Complex, pp 339–345. *In* Transplantation Immunology, F. H. Bach and H. Auchincloss (eds), John Wiley & Sons, New York, NY.

Schook, L. B., M. S. Rutherford, J.-K. Lee, Y.-C. Shia, M. Bradshaw, and J. K. Lunney, 1996. The swine major histocompatability complex, pp 107–122. *In* The Major Histocompatability Complex of Domestic Animal Species, L. B. Schook and S. J. Lamont (eds), CRC Press, New York, NY.

EPIDEMIOLOGY AND CONTROL OF TRICHINAE IN THE NORTHEAST

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OBJECTIVE A: Validate the use of the ELISA test as a tool for identifying trichinae-infested premises.

PROGRESS A: Studies in New England and New Jersey have been undertaken using the ELISA to identify farms where transmission of trichinellosis is occurring. A total of 90 farms have been tested in New England and approximately 60 farms have been tested in New Jersey. Preliminary results suggest that the incidence of trichinellosis has declined since the last regional survey in 1985. Serological positive animals have been confirmed by digestion studies in approximately one-half of the cases. Steps are being taken to determine if the other serological positives reflect exposure to sylvatic types of *Trichinella* which do not infect pigs at a significant level. All positive animals have had infection levels which are of no public health significance (< 1 larvae per g). Studies are underway in Ohio to verify the performance of a commercial ELISA. In experimentally infected pigs, the LMD *Trichinella* Serology Microwell kit performed as-well-as the ARS research ELISA in detecting infected pigs and in reducing background. A comparison of these tests will be published soon (Gamble and Patrascu, 1996). Testing of 12,000 sera using the commercial test kit is ongoing in the Ohio lab and verification is being performed in the ARS, Beltsville lab.

IMPACT/TECH TRANSFER A: The results of this portion of the study will be validation of the commercial test kit for trichinellosis in pigs which can then be used in the industry effort to monitor the production of trichinae-free pigs.

OBJECTIVE B: Associate risk factors with the presence of trichinae in pigs (for on-farm control programs).

PROGRESS B: Data has been acquired from 90 premises tested in New England and approximately 60 premises in New Jersey. Farm data has been entered into EpiInfo and used to evaluate the risk factors for trichinellosis. Preliminary results suggest that primary risk factors are exposure to wildlife and rodents.

IMPACT/TECH TRANSFER B: Risk factor analysis will provide the necessary information for development of criteria under which pigs can be raised trichinae-free.

PUBLICATIONS:

Gamble, H. R. and I. V. Patrascu. 1996. Use of blood and tissue fluids in a serum-based ELISA for swine trichinellosis. *J. Food Prot.* (In press).

Lambillotte, D. and H. R. Gamble. 1997. Evaluation of a rapid ELISA for the detection of serum antibodies in swine to *Trichinella spiralis*. Proceedings of the 9th International Conference on Trichinellosis, Mexico City, Mexico. (In press).

TRICHINAE INSPECTION IN SWINE AND HORSES

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OBJECTIVE A: Provide training and quality control to a program for the inspection of pork and horsemeat for export to the European Union.

PROGRESS A: The ARS administers a training program for the certification of trichinae analysts in swine and horse slaughter facilities. The program is accepted by the European Union and allows U.S. horsemeat and pork to be certified trichinae free for export. Two or more training sessions are held each year for personnel from participating plants to become certified. On a quarterly basis, check samples are prepared and distributed to all certified trichinae analysts for testing. Accurate analysis of these check samples allows for continued certification of these inspectors.

IMPACT/TECH TRANSFER A: The continuation of this program allows U.S. swine and horse slaughter plants to export to European Union countries.

OBJECTIVE B: Provide data supporting the efficacy of current and proposed methods for the inspection of horses and pigs for trichinellosis.

PROGRESS B: Research projects to determine the effectiveness of digestion and serology methods for detection of trichinellosis in pigs and horses have been undertaken by ARS scientists and scientists from Agriculture Canada. Horses were given various doses of *Trichinella spiralis* then slaughtered and tissues and serum tested using methods approved by the EU and USDA. These studies demonstrated that the tongue and masseters were the best muscles to sample using pooled digestion methods and that a 5 or 10 g sample size was required to assure that there was no public health risk associated with digestion negative animals. One g samples, as mandated for inspection of pigs in the EU were not acceptable. Serology testing by ELISA was as effective as the digestion methods for detecting horses with trichinellosis. The results of this study will be used by the USDA and the EU to clearly define the requirements for inspection of horses for trichinellosis. A large study in pigs was undertaken to demonstrate the efficacy of digestion using 1 and 5 g samples. As with the horse study, 1 g samples were not capable of detecting infections below 3–5 larvae per g (LPG). Infections > 1 LPG are considered to be of public health importance. Sample sizes of 5 g was effective in detecting all

infections > 1 LPG. Serology testing was extremely sensitive, but infections were not detected until 21–63 days following infection.

IMPACT/TECH TRANSFER B: These results will be useful for setting inspection requirements for trichinae in pork which will assure public health.

PUBLICATIONS:

Gamble, H. R. 1995. Detection of trichinellosis in pigs by digestion and enzyme immunoassay. *J. Food Prot.* **59**:295–298.

Gamble, H. R., A. A. Gajadhar, and M. B. Solomon 1995. Detection methods for trichinellosis in horses. *J. Food Prot.* **59**:420–425.

PORCINE TOXOPLASMOSIS NATIONAL PREVALENCE

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OBJECTIVE A: Determine the prevalence of toxoplasmosis in swine and develop control procedures.

PROGRESS A: In a serologic survey, 23.9% of 11,842 commercial pigs slaughtered in 1983–84 throughout the United States had antibodies to *Toxoplasma gondii* by the use of a modified agglutination test (MAT). Follow up field studies were initiated in 1992 on swine farms in Illinois to determine risk factors associated with toxoplasmosis in hogs. These results are summarized here.

Field studies on 170 swine farms in Illinois indicated that the prevalence of *T. gondii* is declining (sows 15.1% and feeder pigs 2.3%) compared to the national survey a decade earlier. Analysis of all risk factors indicated that the presence of *T. gondii* infected cats and mice were the two most important risk factors (Dubey *et al.*, 1995, Weigel *et al.*, 1995).

Two strategies to control *T. gondii* infection are being assessed. The first strategy is to vaccinate cats against shedding of oocysts. A *Toxoplasma* vaccine for cats has been developed, using the mutant T-263 strain, which does not produce oocysts. A field trial of the effectiveness of this vaccine in reducing the exposure of finishing pigs to *Toxoplasma* was conducted on 8 swine farms in Illinois. In previous field investigations these farms were identified to have high seroprevalence rates for *Toxoplasma* infection in swine and cats. Each farm was visited 3 times each year in 1994 and 1995, and once in 1996. Cats were trapped and inoculated orally with the *Toxoplasma* vaccine. Blood samples were collected from pigs, cats, and mice and tested for antibodies to *Toxoplasma*, using the MAT. Fecal samples also were obtained from cats for detection of shedding of oocysts. The impact of vaccination of cats upon *Toxoplasma* seroprevalence rates in previously unvaccinated cats, sows, finishing pigs, and mice is being evaluated.

The second strategy is to vaccinate pigs against *T. gondii*. For this, a natural mutant of the RH strain has been discovered. Intramuscular inoculation of pigs with the live vaccine strain tachyzoites immunizes the pigs but the parasite is killed in pig tissues within 2 weeks of vaccination. When vaccinated pigs were challenged with 100,000

live virulent oocysts, *Toxoplasma* was not found in tissues. The mechanism of this protection is being studied in both outbred and inbred pigs. During this investigation it was discovered that pigs inoculated with 1 live oocyst developed patent *T. gondii* infections (Dubey *et al.*, 1996). Another approach to immunizing animals against *T. gondii* is to vaccinate them with irradiated oocysts. For this, *T. gondii* oocysts were irradiated at 0.10, 0.20, 0.25, 0.30, 0.40 and 0.5 KGy of Cesium^{137}. *Toxoplasma* oocysts were killed by 0.25 KGy irradiation. However irradiated oocysts retained immunogenicity. Mice fed oocysts irradiated at 0.40 KGy survived lethal challenge with *T. gondii*. Studies are now in progress to determine immunogenicity of irradiated oocysts in pigs.

IMPACT/TECH TRANSFER A: The results of this study will be useful for pig farmers to institute management changes in order to produce *Toxoplasma* free pigs.

PUBLICATIONS:

Dubey, J. P., M. C. Jenkins, D. W. Thayer, O.C.H. Kwok, and S. K. Shen. 1996. Killing of *Toxoplasma gondii* oocysts by irradiation and protective immunity induced by vaccination with irradiated oocysts. *J. Parasitol.* (In press).

Dubey, J. P., J. K. Lunney, S. K. Shen, O.C.H. Kwok, D. A. Ashford, and P. Thulliez. 1996. Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs. *J. Parasitol.* **82**:438–443.

Dubey, J. P., R. M. Weigel, A. M. Siegel, P. Thulliez, U. D. Kitron, M. A. Mitchell, A. Mannelli, N. E. Mateus-Pinilla, S. K. Shen, O.C.H. Kwok, and K. S. Todd. 1995. Sources and reservoirs of *Toxoplasma gondii* infection on 47 swine farms in Illinois. *J. Parasitol.* **81**:723–729.

Weigel, R. M., J. P. Dubey, A. M. Siegel, U. D. Kitron, A. Mannelli, M. A. Mitchell, N. E. Mateus-Pinilla, P. Thulliez, S. K. Shen, O.C.H. Kwok, and K. S. Todd. 1995. Risk factors for transmission of *Toxoplasma gondii* on swine farms in Illinois. *J. Parasitol.* **81**:736–741.

TOXOPLASMA GONDII RECOMBINANT ANTIGEN

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OBJECTIVE A: Develop a low-cost assay to detect antibodies to *Toxoplasma*.

PROGRESS A: The objective of this project is to develop a reliable, sensitive, specific and simple assay to detect antibodies to *Toxoplasma gondii* in naturally infected animals. In order to have a reliable antigen available in large quantities, 6 recombinant antigens of *T. gondii*, H11, H4, B427, V22, C55, and C51, were assessed for application in an enzyme-linked immunosorbant assay (ELISA) tests for toxoplasmosis in swine. The antigens were evaluated with sera from experimentally infected, young pigs and with sera from slaughter sows which had been bioassayed for *T. gondii*. Recombinant antigen ELISAs demonstrated a sharp rise in antibody response very early after infection in sera from experimentally-infected pigs. In comparison to ELISAs with native antigen, recombinant antigens were more reactive with early post-infection sera, and less reactive with late (chronic) infection sera. For slaughter sows, detection of *Toxoplasma gondii* in heart tissue by bioassay was the gold standard used to assess ELISA results. ELISAs, using a combination of B427 and V22 antigens, detected 23 of 37 bioassay positive sows (62% sensitivity) with 4 false positive reactions (92.5% specificity). Native antigen ELISA detected 18 of 37 bioassay positive sows (48.6% sensitivity) with 1 false positive (98% specificity). These results are encouraging and efforts are being made to test more recombinant antigens.

In order to develop serologic tests for the detection of chronic infection in pigs, antibody titers to *Toxoplasma gondii* were determined in 16 pigs orally inoculated with 1000 or 10,000 oocysts of one of the 4 strains (GT-1, ME-49, TS-2 and TC-2) of *T. gondii*. Pigs were euthanized postinoculation day (PID) 103 to 875 and their tissues were bioassayed for *T. gondii*. Antibody titers were measured in the modified agglutination test (MAT) using formalin-preserved (test A) or acetone-preserved (test B) tachyzoites, the latex agglutination test (LAT), the indirect hemagglutination test (IHA), the ELISA, and the Sabin-Feldman dye test (DT). *Toxoplasma gondii* was isolated from all but 2 (one with GT-1 strain and one with TC-2 strain) of the inoculated pigs. Results of the serologic tests varied by the test used, by the strain of *T. gondii*, and from pig to pig within groups. One pig inoculated with the TC-2 strain was considered to be not infected because it remained seronegative in all tests and *T. gondii* was not isolated from its tissues by bioassay. The IHA and LAT did not produce consistently positive results with infected

pigs and 2 pigs remained seronegative (< 1:64) in both tests. At the time of necropsy, IHA titers had declined to < 1:64 in 5 pigs and LAT titers had declined to < 1:64 in 4 pigs. The MAT (test A) and the ELISA detected antibodies in all infected pigs, but the ELISA did not detect antibody at the time of necropsy in one pig. Antibody titers peaked earliest in the DT. Antibody titers in the MAT (test B) peaked at 2560 in all seropositive pigs by 6 weeks postinoculation but declined to < 1:160 by 15 weeks postinoculation; this test may be useful in determining recency of *T. gondii* infection in pigs. Overall, the MAT (test A) gave the most consistant results.

IMPACT/TECH TRANSFER A: These results will be useful for industry to produce and market reagents for serologic testing of pigs for *Toxoplasma* infection.

PUBLICATIONS:

Dubey, J. P., C. D. Andrews, P. Thulliez, P. Lind, and O.C.H. Kwok. 1996. Long-term humoral antibody responses by various serologic tests in pigs orally inoculated with oocysts of four strains of *Toxoplasma gondii*. *Vet. Parasit.* (In press).

CONTROL AND PREVENTION OF *CRYPTOSPORIDIUM PARVUM* INFECTION

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OBJECTIVE A: Determine the potential for transmission of *Cryptosporidium parvum* from food animals to humans via contaminated water or milk.

PROGRESS A: We tested the efficacy of commercial pasteurization conditions to destroy infectivity of *Cryptosporidium parvum* oocysts in water or whole milk. We found that the time and temperature conditions of commercial pasteurization were sufficient to kill high concentrations of *C. parvum* oocysts seeded into water or whole milk.

IMPACT/TECH TRANSFER A: These findings indicate that the threat of on-farm contamination of milk products with *C. parvum* can be effectively eliminated by adherence to standard commercial pasteurization conditions. In addition, proper pasteurization of commercially bottled water can provide a *Cryptosporidium*-free product for use by humans at high risk for *C. parvum* infection, *i.e.*, infants and immunocompromised individuals.

Part II. PATHOGEN CONTROL DURING SLAUGHTERING AND PROCESSING

TREATMENT AND REUSE OF WATER IN COMMERCIAL FOOD PROCESSING OPERATIONS

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OBJECTIVE A: Investigate systematically the causal relationship between chlorination and mutagen formation in food processing water and determine the specific mutagens formed upon chlorination.

PROGRESS A: The research addresses the problem of deleterious chemical byproduct formation by chlorine used for sanitation of poultry chiller water (PCW). A common biological test for chemical mutagens, the Ames *Salmonella* bioassay, indicates that PCW mixtures become mutagenic only when chlorination levels are increased above the levels authorized for use in poultry plants. In past experiments we used simulated poultry chiller water with an elevated level of 1600 ppm chlorine to facilitate molecular structure identification of the disinfection byproducts. We detected and identified a single tetrachloroimide compound which accounted for a significant portion of the mutagenicity. The same mutagenic chloroimide has now been detected at 200 ppm of chlorine after laboratory chlorination of unchlorinated PCW obtained from a local processing plant. A quantitative dose-response of chloroimide concentration vs. chlorination level in PCW is being completed as a final step in establishing a risk-assessment basis for mutagen formation for PCW. Using computational methods we have been able to correlate two calculated electronic properties, unoccupied orbital energy and electron affinity, with the Ames mutagenicity results. Interestingly, the PCW-derived chloroimide follows the same predictive model as the mutagen "MX", a potent mutagen formed during chlorination of drinking water. This discovery links the risk assessment process for poultry chlorination to investigations underway elsewhere on the toxicology of disinfection byproducts in drinking water.

IMPACT/TECH TRANSFER A: We have shown that no mutagens are formed when the chlorination levels are held under authorized levels. The calculated electronic

properties of the mutagenic chloroimide correlate with the Ames test results, and the chloroimide follows the same predictive model as the mutagen 'MX'. This result links the risk assessment process for the mutagenic chloroimide in PCW with those studies elsewhere for disinfection byproducts in drinking water.

OBJECTIVE B: Develop new disinfectants, such as chlorine dioxide, for the disinfection of meat and poultry processing water.

PROGRESS B: Our research on the chemical disinfection of chlorine dioxide in poultry chiller water has led to the approval of its use by FDA. Subsequently, 10 poultry processors supported by 2 chlorine dioxide suppliers applied for approval from FSIS for in-plant testing. The tests have achieved limited success. Several tests were suspended without reaching completion due to generation of a strong irritating odor by the chlorine dioxide treatment. We believe the odor problem was from chlorine dioxide overdose. However, it cannot be corrected easily because there is no adequate method for the determination and monitoring of chlorine dioxide, and no preventive measure was devised for the potential escape of chlorine dioxide from the chiller. Since chlorine dioxide is potentially a safer and more efficient alternative to chlorine in poultry processing, we have initiated research to develop the methodology for the accurate determination of chlorine dioxide in processing water.

The chlorine dioxide derivatives, chlorate, chlorite and chloride, were successfully quantified at less than 1 ppm by an HPLC method. Unfortunately, the method cannot be used to measure chlorine dioxide and hypochlorite directly, because they will be converted to chlorite and chloride, respectively on the analytical columns. The three stable chlorine-containing ions, but not chlorine dioxide, also can be quantified by capillary electrophoresis. Several new approaches to the analysis of chlorine dioxide are being evaluated. These include the use of capillary zone electrophoresis with open tubular silica columns and modified columns, and micellar electrokinetic chromatography with surface active agents (function), such as sodium dodecyl sulfate.

In the current practice of disinfection a large portion of the chlorine dioxide added was consumed by the organic materials in the chiller water. This not only reduced disinfection efficiency, but also resulted in chemical adulteration. To overcome these undesirable results, we are investigating the possibility of disinfecting chicken carcasses with chlorine dioxide directly. A treatment chamber that specifically addresses workers' safety, was designed and is being constructed. Chicken carcasses will be tested for disinfection efficacy and residual concentration under various treatment conditions.

IMPACT/TECH TRANSFER B: Due to the strong irritating odor of chlorine dioxide earlier tests using this chemical were abandoned. We have designed a new chlorine dioxide disinfecting chamber for chicken carcasses. This new approach should overcome the undesirable results with chlorine dioxide, and address worker's safety.

OBJECTIVE C: Develop physical treatment methods for the reuse of water and brine in meat and poultry processing.

PROGRESS C: John R. Daily Inc., Missoula, MT, a bacon manufacturer, and Star Filter Co., Timmonsville, SC, an equipment supplier, have agreed to collaborate with WRRC to conduct an in-plant test for the physical treatment of brine for reuse. The treatment uses diatomaceous earth impregnated paper that uniformly excludes any particles including bacteria exceeding 0.3 μm. The process has been demonstrated at WRRC to be microbiologically safe and this has been verified in limited runs at a commercial site. The protocol of the proposed operation is currently being reviewed by FSIS.

A systematic study investigated the effects of proteins and lipids on ceramic microfiltration. The filtration rate (flux) of protein solutions made from bovine serum albumen (BSA) was found to be pH dependent with the highest rate at pH 10. Filtration rate decreased with increasing acidity. The flux of lipid/water suspensions was dependent on the lipid type. Fish-oil suspension had the highest flux, followed by chicken oil and lard. Increasing the operating temperature from 25 to 50 °C had no significant effect on fish-oil suspension, but increased the filtration rate of chicken oil suspension considerably. These results suggested that the filtration flux depends on the physical state of the lipid since fish-oil is a liquid in this temperature range and chicken oil becomes liquid at 50 °C.

IMPACT/TECH TRANSFER C: The filtration of brine allowing the exclusion of bacteria would allow brine to be reused by the processing physicality. Showing that a temperature increase to 50 °C would greatly improve the filtration of chicken oil suspensions should help improve our in-plant filtration processes. This would mean a savings to the poultry industry, and also produce less environmental impact from waste used brine.

PUBLICATIONS:

Haddon, W. F., R. G. Binder, R. Y. Wong, L. A. Harden, R. E. Wilson, M. Benson, and K. L. Stevens. 1996. Potent bacterial mutagens produced by chlorination of simulated poultry chiller water. *J. Agric. Food Chem.* **44**:256–263.

ENGINEERING INNOVATIONS AND MICROBIOLOGICAL DEVELOPMENTS TO REDUCE THE MICROBIAL CONTAMINATION OF PROCESSED POULTRY

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OBJECTIVE A: Pre-evisceration treatments; spray scalding.

PROGRESS A: Research on spray scalding continues under a CRADA with Johnson Food Equipment CO., Kansas City, MO. Initial experiments were conducted to optimize heat control of the spray. Subcutaneous temperatures of carcasses were profiled for two immersion scald (52 and 56.5 °C) and three spray scald temperatures (60, 65, and 70 °C). Eight thermocouple locations were used to monitor the subcutaneous (muscle surface) temperature during the 2 min immersion scald or 30 sec spray scald. Thermocouple locations were the right and left breast, right and left thigh, under the wings, lower back, and upper back between the wings. The profiles were used to determine the optimum spray temperature for defeathering without denaturing the surface of the muscle. Microbiological comparisons were made using the optimum spray temperature and compared to immersion scalding at 56 °C for 2 min. No statistical differences were found in the total plate counts or *Enterobacteriaceae* counts due to the method of scalding. During the next phase of work, the ability of various organic bactericides combined with the picker spray water at various water temperatures to reduce microbial counts will be determined.

IMPACT/TECH TRANSFER A: Successful implementation of spray scalding will be used by the poultry industry to reduce cross-contamination among carcasses and reduce the amount of water needed in scalding. Actual transfer of the technology will require at least one more year of pilot plant research prior to commercial testing.

OBJECTIVE B: Test computer simulations of water mixing and bacterial residence times in poultry scald tanks.

PROGRESS B: Previous work led to the development of a computer program that can predict mixing patterns of bacteria suspended in scald tank water, a necessary step to be able to predict bacterial residence times and death in the hot water. A chain conveyor was added to a pilot plant scald system to permit moving simulated carcasses through the tank.

Movement of dye through the model system was correctly predicted by the computer program with simulated carcasses with size equivalent to 5% or 26% of tank cross-sectional area. The simulation program can be used to predict water mixing and bacterial residence times in scald tanks

IMPACT/TECH TRANSFER B: This project is now at a point where field trials are needed to test the computer simulation under actual industrial conditions. Trials will begin as soon as an industrial cooperator is identified. Successful commercial test, would allow the computer model to be an important part in defining processing parameters required to increase bacterial mortality through improved scald tank design and operation.

OBJECTIVE C: Pre-evisceration treatments; chemical spray during feather removal.

PROGRESS C: The multiple machines used to remove feathers (pickers) have long been considered one of the primary causes of carcass contamination and/or cross-contamination during processing. Acetic acid or hydrogen peroxide were added to the picker water spray during defeathering. Either water, a 1% acetic acid solution or, 0.5, 1, or 1.5% solutions of hydrogen peroxide were sprayed on broiler carcasses during defeathering and total plate counts made for microbiological evaluations of the treatments. After picking, the neck and feet were removed and carcass vents were blocked with cotton plugs to prevent spillage of fecal material during the whole carcass rinse procedure. The carcasses were evaluated for total aerobic organisms and for visual skin changes due to the chemical treatments. Carcasses subjected to the acetic acid treatment had significantly lower total plate counts (0.6 $\log_{10}$ CFU/mL) and there was no visual difference when compared to the water sprayed controls. The hydrogen peroxide treated carcasses showed no differences microbiologically, but the carcasses appeared to be bleached and bloated. Work is continuing with the acid and a strong base at elevated temperatures to try and reach even higher reductions in microbial loads.

IMPACT/TECH TRANSFER C: The addition of the GRAS chemical acetic acid during defeathering should reduce microbial counts and incidence of enteric pathogens exiting the pickers without altering carcass skin appearance. Reduction or elimination of cross-contamination with the acid treatment is a real possibility and would drastically reduce the incidence of enteric pathogens exiting the defeathering equipment.

OBJECTIVE D: Pre-evisceration treatments; Acid/Base Dips

PROGRESS D: The effectiveness of low pH and high pH chemicals used alone and in tandem on reducing the microbial loads on New York dressed carcasses was tested. Carcasses were dipped into either an acid (pH < 3) then a base (pH > 11) or a base and then an acid. Carcasses were processed under commercial conditions and the vent plugged after picking to prevent fecal contamination during the whole carcasses rinse procedure. After plugging, carcasses were submerged in either an acetic acid solution for

15 sec, or a trisodium phosphate solution for 15 sec. Carcasses were then microbiologically sampled using the whole carcass rinse procedure. Other carcasses were treated with combinations of either an acid then base dip or a base then acid dip. Results showed a significant reduction in total plate counts for all treatments when the acetic acid was the only or final dip. No microbiological differences were seen when the trisodium phosphate was the only or final dip. Total aerobic plate count reductions ranged from 0.42 to 0.65 Log_{10} CFU/mL depending on the treatment. Research will continue on the use of these chemicals at various temperatures in an effort to find optimum conditions for use in poultry processing plants.

IMPACT/TECH TRANSFER D: The use of acetic acid, possibly in combination with trisodium phosphate, on processed poultry carcasses could prove to be an effective intervention step for improving the overall microbiological quality of processed poultry.

OBJECTIVE E: Determine the relationship between aerobic plate count and the likelihood of pathogens on broiler carcasses.

PROGRESS E: The slowness of traditional microbiological methods for detecting human pathogens has led to a search for possible substitute or "index" bacteria. Testing for aerobic bacteria, for instance, is considerably faster than testing for pathogens. Microbiological sampling of broiler carcasses at three locations in a processing plant failed to demonstrate any predictive relationship between aerobic plate count and either salmonellae or *Campylobacter*. No APC-pathogen relationship was found even when the analysis included the "dirtiest" carcasses (those with APC values more than one standard deviation above the mean).

IMPACT/TECH TRANSFER E: Aerobic bacteria are not suitable index organisms for salmonellae or *Campylobacter*. Development of rapid microbiological testing methods still needs to focus directly on the pathogens.

OBJECTIVE F: Construction of a laboratory scale model brushing machine.

PROGRESS F: Logistical problems with the cooperating company prevented installation of the original brushing machine in a commercial processing plant. Accordingly, a laboratory scale version of the brush machine was designed and fabricated in the machine shop at the Richard B. Russell Research Center. The laboratory scale brusher will be used in testing for the microbiological effects of scrubbing the vent and breast area of poultry carcasses between the stages of a multi-stage scalder. Initial studies will be conducted in a pilot plant environment. Laboratory trials designed to test the machine are presently in the planning stages.

IMPACT/TECH TRANSFER F: Removal of organic material from the carcass beween stages of a multi-stage scalder will result in less fecal material and dust entering the final

stages of scalding. The reduced organic load should result in lower microbiological counts and numbers of enteric pathogens on carcasses. This could be used as an intervention step in an overall HACCP program for poultry processing.

OBJECTIVE G: Alternative means to remove feathers post-mortem from poultry carcasses.

PROGRESS G: Immersing carcasses in hot water to lessen feather retention force followed by 4 or 5 picking machines equipped with rubber fingers to remove feathers has been a standard procedure in the poultry industry for many years. Ante-mortem electrical and CO_2 stunning did not result in significant alteration of feather retention force post-mortem. Transection of the spinal cord immediately following stunning eliminated the peri-mortem death struggle, but did not alter feather retention force. At all time periods sampled, feathers extracted perpendicular to the carcass surface consistently required less force (9 to 29%) to extract than those oriented parallel to the carcass.

IMPACT/TECH TRANSFER G: Results indicate that brain-spinal cord integration is not necessary to retain post-mortem feather retention force. The consistently lower force required to remove feathers oriented perpendicular to the carcass indicate that the angle of feather removal be evaluated in the design of commercial poultry defeathering equipment.

PUBLICATIONS:

Lyon, C. E. and J. A. Cason. 1995. Effects of water chilling on objective color of bruised and unbruised broiler tissue. *Poult. Sci.* 74:1894–1899.

Reduction of Bacterial Contamination and Pathogen Load During Poultry Processing

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OBJECTIVE A: Study the formation and composition of biofilms on processing plant surfaces.

PROGRESS A: Baseline studies to describe the formation of biofilms and the potential of pathogens to become a problem within the biofilms in the processing plant environment were continued. Whole carcass rinse samples were collected from a commercial broiler processing plant after the New York rinse. Attachment of bacteria from the whole carcass rinse to rubber picker finger material was significantly less than attachment to stainless steel, polyethylene, or belt webbing surfaces. Methodology and statistical techniques were developed to assess the repeatability and reproducibility of the protocol. Preliminary results were confirmed in subsequent tests by using the same sample for spectrophotometry and electron microscopy. Studies were initiated to characterize the growth and attachment properties of pathogens from the poultry processing environment.

IMPACT/TECH TRANSFER A: Food safety could be enhanced by increasing the use of materials that do not support growth and attachment of microorganisms while decreasing the use of materials that enhance growth and attachment. Identifying the factors that play a role in pathogen attachment is a necessary step toward determining the relative importance to food safety of pathogens found in the poultry processing plant.

OBJECTIVE B: Develop methods of preventing the formation of or removing, biofilms on processing plant surfaces so as to allow efficacious cleaning and sanitizing.

PROGRESS B: Traditional or standard test organisms used in clinical and hospital tests are not relevant for use as "indicator" organisms to test disinfectants or sanitizers against biofilms found in the food industry. Biofilms are known in general to be more resistant to disinfectants than planktonic organisms of laboratory cultures of single organisms. In addition, the resistance to disinfectants of newly identified organisms of concern to food manufacture has not been documented. Initial evaluation of possible protocols for testing the resistance of a mixture of organisms found in the whole carcass rinse to a range of disinfectants and sanitizers commonly used in the food industry has begun.

IMPACT/TECH TRANSFER B: Inhibiting bacterial attachment will enhance food safety by preventing the increase in bacterial numbers necessary for biofilm formation. Finding the least amount of treatment necessary to effectively inhibit biofilms will be economical for the industry and consumers as-well-as reduce the impact of agriculture on the environment.

PUBLICATIONS:

Arnold, J. W. and P. S. Holt. 1996. Cytotoxicity in chicken alimentary secretions as measured by a derivative of the tumor necrosis factor assay. *Poult. Sci.* 75:329–334.

CONTROL OF PATHOGENIC AND SPOILAGE BACTERIA ON RED MEAT (Carcass Washing Systems)

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OBJECTIVE A: Determine the effect of steam-vacuuming or hot water washes on the microbial ecology of beef carcasses.

PROGRESS A: Bacterial populations on beef carcass surfaces were monitored immediately and over time under vacuum packaged refrigerated storage following hot water washes (W) delivered through a beef carcass wash cabinet or application of steam-vacuum (SV). Fresh, unaltered bovine feces spiked with antibiotic resistant strains of *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes* were used to inoculate beef carcass surface tissues prior to W or SV treatment.

All treatments were immediately equally effective as is indicated by bacterial populations immediately following any of the treatments ($P > 0.05$); however, the combination of SV followed by W consistently produced arithmetically greater bacterial reductions. *E. coli* O157:H7 was initially reduced by as much as 3.4 $\log_{10}$ CFU/cm^2 and did not grow to the original inoculation levels for the duration of the experiment. In general, all treatments produced initial reductions of up to 2.7 $\log_{10}$ CFU/cm^2 for APC, lactic acid bacteria, and *L. innocua*, but by 14 days bacterial numbers had increased to levels of $\geq$ 7 $\log_{10}$ CFU/cm^2. Vegetative counts of *C. sporogenes* were initially reduced by as-much-as 3.4 $\log_{10}$ CFU/cm^2 and numbers continued to decline for the duration of the study. These results indicate that the use of W and SV effectively reduces bacterial populations from beef carcass tissue immediately after treatment. Additionally, storage of treated tissue up to 21 days at 5 °C did not appear to offer any competitive advantage to potentially pathogenic microorganisms. (STATUS: Completed)

IMPACT/TECH TRANSFER A: The USDA-FSIS used studies conducted at Clay Center to help determine the efficacy of using steam vacuum systems in beef slaughter facilities. As a result of these and other in-plant studies, the steam-vacuum system can now be used in an inspected slaughter plant without prior approval from FSIS. Steam-vacuum systems have been successfully incorporated into all large and most smaller beef slaughter plants.

OBJECTIVE B: Determine the effects of acetic, lactic acid and trisodium phosphate (TSP) on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua, and Clostridium sporogenes.*

PROGRESS B: Following chemical antimicrobial treatment, the microbial profiles of inoculated beef carcass tissue (BCT) were monitored during refrigerated vacuum packaged storage. An industrial spray wash cabinet (W) delivered water, 1.5 and 3.0% lactic (LA) or acetic (AA) acid, or 12% TSP washes. Fresh unaltered bovine feces spiked with antibiotic resistant strains of *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes* were used to inoculate BCT prior to all treatments. The effect of treatments on bacterial populations was tracked by monitoring levels of the marked bacteria along with mesophilic aerobic bacteria (APC), lactic acid bacteria (LAB), and pseudomonads for up to 21 days at 5 °C storage.

Initial APC levels of approximately 5.6 $\log_{10}$ CFU/cm^2 were reduced 1.3–2.0 $\log_{10}$ CFU/cm^2 by LA, AA, and TSP treatments. Marked bacteria were reduced to < 1.3 $\log_{10}$ CFU/cm^2, remaining that way throughout 21-day storage. TSP treatments were not different in effectiveness from acids for controlling growth of *E. coli* O157:H7 and *C. sporogenes*, but were less effective for APC, *L. innocua* or LAB. The aerobic bacteria, *L. innocua*, and LAB had counts $\geq$ 7 $\log_{10}$ CFU/cm^2 by 7 days in all but one case and by 14 days all had counts > 7 $\log_{10}$ CFU/cm^2 on the untreated controls and water washed samples.

Treatments generally added a degree of safety regarding the foodborne pathogens and pathogen models used for the present study when beef tissue was stored up to 21 days and in no case did the treatments appear to offer any competitive advantage to select microorganisms on BCT. (STATUS: Completed)

IMPACT/TECH TRANSFER B: Since beef consumption does not occur until some period of time after the carcass is processed, it is important to track bacteria of interest well past the point of initial reductions when determining food safety advantages of these interventions. The incorporation of various antimicrobials as total carcass treatments is presently in limited use by the beef processing industry. With the increased emphasis the new HACCP based inspection system places on the microbial profile of raw beef carcasses, processors are aggressively evaluating or re-evaluating the feasibility of many new and older total carcass antimicrobial treatments. This information has been disseminated via presentations made to professional and industrial groups and is submitted for publication.

OBJECTIVE C: Determine various parameters that may affect removal of *E. coli* O157:H7 and fecal contamination on beef by spray washing.

PROGRESS C: A series of progressive experiments was conducted with a model carcass washer using tap water and 2% acetic acid sprays to determine if tissue type, inoculation

menstruum, bacterial level, or spray temperature affect removal of bacteria from beef carcass tissue during spray washing.

Type of tissue surface. Pre-rigor or post-rigor frozen lean beef carcass surface tissue (BCT) was inoculated with bovine feces and subjected to spray washing (15 sec, 56 °C) with water or acetic acid. Spray washing with either compound resulted in similar bacterial populations for pre-rigor and post-rigor BCT; however, remaining bacterial populations from spray-treated post-rigor, frozen BCT were significantly ($p < 0.05$) less than the other two tissue types.

Inoculating menstruum. Pre-rigor, lean BCT was inoculated with *Escherichia coli* O157:H7 suspended in bovine feces or physiological saline and spray washed with water or acetic acid. Regardless of menstruum, feces or saline, bacterial populations were reduced to similar levels with acid sprays.

Inoculum levels. Beef carcass tissue was contaminated to different levels (7, 5, 3, and 1 $\log_{10}$ CFU/ cm^2). Spray washes with acetic acid reduced the level of the pathogen *E. coli* O157:H7 in feces to 2.51 and 0.3 $\log_{10}$ CFU/cm^2 when initial bacterial levels were 7 and 5 $\log_{10}$ CFU/cm^2. Residual *E. coli* O157:H7 were not detectable from initial inoculum levels of 3 and 1 $\log_{10}$ CFU/ cm^2.

Wash temperature. Water or acetic acid, ranging from 30 to 70 °C, was applied to beef tissue contaminated with *E. coli* O157:H7 in feces. Remaining bacterial populations were not different between the water treatments or between the acid treatments at any temperature. (STATUS: Completed)

IMPACT/TECH TRANSFER C: This study demonstrates that while a parameter such as bacterial inoculum level may affect the efficacy of spray washing with organic acids; the following parameters had no affect: inoculation menstruum, tissue type, and spray temperature.

OBJECTIVE D: Determine whether an edible film can act as a carrier or delivery system for bacteriocins to ground beef.

PROGRESS D: Lean and adipose beef carcass tissues were inoculated with the meat spoilage bacterium, *Brochothrix thermosphacta* (Bth.), then left untreated (U), treated with 100 µg/mL nisin (N), calcium alginate (A), or 100 µg/mL nisin immobilized in a calcium alginate gel (AN). Treated tissues were aseptically processed into ground beef and populations of *B. thermosphacta* and nisin activity were determined at 0, 1, 7, and 14 days.

Nisin in a calcium alginate gel reduced the levels of Bth. to undetectable levels (< 1.3 $\log_{10}$ CFU/g) at day 0, while populations of U-, A-, and N-treated ground beef were 3.24, 3.17, and 2.8 $\log_{10}$ CFU/g, respectively. In contrast to high nisin titers from AN-treated ground beef at day 0, nisin titers were undetectable in N-treated ground beef. By day 7, *B.*

thermosphacta had grown to 7.18, 7.04, and 6.92 $\log_{10}$ CFU/g in U-, A-, or N-treated ground beef, respectively, while AN-treated ground beef exhibited maximal populations of 6.56 $\log_{10}$ CFU/g. By day 7, nisin titers from AN-treated ground beef were diminished considerably. At day 14 of the study, all treatments exhibited bacterial populations > 7 $\log_{10}$ CFU/g and nisin titers were virtually undetectable in any of the ground beef samples; however, *B. thermosphacta* never grew to levels of untreated ground beef. (STATUS: Completed)

IMPACT/TECH TRANSFER D: This study demonstrates that immobilization of a bacteriocin on the surface of beef within an edible gel was a more effective delivery system for a bacteriocin into ground beef than direct application.

OBJECTIVE E: Determine if application of hydrogen peroxide, alone or in combination with sodium bicarbonate or acetic acid, is effective for reducing *E. coli*, *Listeria* spp., and *Salmonella* spp. from beef surfaces.

PROGRESS E: Adipose and lean beef carcass tissues were inoculated with sterile feces containing *E. coli*, *Listeria innocua*, or *Salmonella wentworth*. A model carcass washer (80 psi, 15 sec, 25 °C) was used to apply water, 1% acetic acid, 3% hydrogen peroxide, 1% sodium bicarbonate, or a combination of acetic acid/hydrogen peroxide or sodium bicarbonate/hydrogen peroxide. In addition to bacterial populations, samples were analyzed for Hunter color, residual hydrogen peroxide, and surface pH immediately after treatments and after 24 hr refrigerated storage.

Of the treatments investigated, a combination of acetic acid/hydrogen peroxide was found to be the most effective, resulting in reductions of > 99.9% for *E. coli, L. innocua,* and *S. wentworth* on either tissue type. Hunter color analyses immediately following spray washing with hydrogen peroxide indicated some bleaching of the beef tissue, but after 24 hrs refrigerated storage, this bleaching effect was not detectable. Similarly, residual hydrogen peroxide was detected immediately after spray washing, but not after 24 hrs refrigerated storage. Acetic acid-treated tissues exhibited lower surface pH values on both tissue types immediately after spray washing and after 24 hrs refrigerated storage. (STATUS: Completed)

IMPACT/TECH TRANSFER E: Based on the results of this study, spray wash treatments consisting of the right combination of antimicrobials can be more effective than single applications for reducing undesirable bacteria on beef surfaces.

PUBLICATIONS:

Cutter, C. N. and W. J. Dorsa. 1995. Chlorine dioxide spray washes for reducing fecal contamination on beef. *J. Food Prot.* **58**:1294–1296.

Cutter, C. N., W. J. Dorsa, and G. R. Siragusa. 1996. Application of Carnatrol® and Timsen® to decontaminate beef. *J. Food Prot.* (In press).

Cutter, C. N. and G. R. Siragusa. 1995. Population reductions of gram negative pathogens following treatments with nisin and chelating agents under various conditions. *J. Food Prot.* **58**:977–983.

Cutter, C. N. and G. R. Siragusa. 1995. Treatments with nisin and chelators to reduce *Salmonella* and *Escherichia coli* on beef. *J. Food Prot.* **58**:1028–1030.

Cutter, C. N. and G. R. Siragusa. 1996. Reductions of *Listeria innocua* and *Brochothrix thermosphacta* on beef following nisin spray treatments and vacuum packaging. *Food Microbiol.* **13**:23–34.

Cutter, C. N. and G. R. Siragusa. 1996. Reduction of *Brochothrix thermosphacta* on beef surfaces following immobilization of nisin in calcium alginate gels. *Lett. Appl. Microbiol.* **23**:9–12.

Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1996. Effectiveness of a steam-vacuum sanitizer for reducing *Escherichia coli* O157:H7 inoculated to beef carcass surface tissue. *Lett. Appl. Microbiol.* **23**:61–63.

Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1996. Effects of steam-vacuuming and hot water spray washes on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*. *J. Food Prot.* (In press).

Dorsa, W. J., C. N. Cutter, G. R. Siragusa, and M. Koohmaraie. 1996. Microbial decontamination of beef and sheep carcasses by steam, hot water spray washes, and steam-vacuum sanitizer. *J. Food Prot.* **59**:127–135.

Dorsa, W. J., G. R. Siragusa, C. N. Cutter, E. D. Berry, and M. Koohmaraie. 1996. Efficacy of using a sponge sampling method to recover low levels of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and aerobic bacteria from beef carcass surface tissue. *Food Microbiol.* (In press).

CONTROL OF PATHOGENIC AND SPOILAGE BACTERIA ON RED MEAT (Reconditioning Accidentally Contaminated Meat and Poultry)

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OBJECTIVE A: Determine the efficacy of using a sponge sampling method to recover low levels of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and aerobic bacteria from beef carcass surface tissue.

PROGRESS A: Recovery of aerobic bacterial populations as-well-as low levels of *Escherichia coli* O157:H7 and *Salmonella typhimurium* from beef carcasses was determined by sponge (SP) and excision (EX) sampling during the course of three separate studies.

Study 1. Samples were taken from three points on the processing line: 1) pre-wash, 2) post-wash, and 3) after 24 hrs in the chill cooler. Sponge sampling recovered fewer total aerobic bacteria from beef carcasses on a processing line than EX; however, the difference between EX and SP was similar at all sampled process points. Both sampling methods recovered higher levels of total aerobic bacteria from carcasses after a 24 hr chill period. Both methods also recovered low levels of *E. coli* and coliforms on beef carcasses.

Study 2. Both SP and EX recovered antibiotic-marked strains of *E. coli* O157:H7 and *Salmonella typhimurium* from beef carcass tissue surface at an inoculation level of approximately 1 CFU/100 cm^2. Recoveries from pre- or post-24 hr chilled tissue were similar for both SP and EX. Sponge sampling appears to be an adequate sampling method for recovery of low levels of pathogens from surfaces of beef carcasses regardless of location within the process.

Study 3. The third study demonstrated that the freezing and storage of SP samples at –20 °C significantly decreased recovery of *S. typhimurium* from beef tissue when present at low levels (≤ 10 CFU/100 cm^2). (STATUS: Completed)

IMPACT/TECH TRANSFER A: While excision is the most effective method for sampling beef carcasses, the USDA-FSIS needed a more rapid, non-destructive, representative sampling method to incorporate into the new 'Mega-Reg". Consequently, the sponge carcass sampling method was developed and then validated. This method has been incorporated into the new inspection regulations (see code of Federal Regulations

(CFR), 9CFR, Part 304 *et al.*, Pathogen Reduction, Hazard Analysis and Critial Control Point (HACCP) Systems; Final Rule).

OBJECTIVE B: Develop a simple and representative purge sampling method to detect total aerobic bacteria and *Escherichia coli* O157:H7 in raw beef combos (bulk lean trimmings).

PROGRESS B: The purge from beef combos was tested as a means for representatively sampling the microbial content of this raw product. Purge was sampled from beef combos that had been inoculated with bovine feces (Phase 1) or inoculated with an antibiotic resistant *Escherichia coli* O157:H7 (Phase 2). The purge from both phases was assayed for bacteria using culture methods. Data (n = 82) from phase one indicated a strong correlation (r = 0.94) between the total aerobic bacteria counts derived from the purge samples of a combo of beef and the total aerobic bacteria present in a rinse sample of the entire combo of beef.

Marked *E. coli* O157:H7 was retrievable from raw meat combos after 24 hrs regardless of the location of the inoculated pieces of meat within the 75 cm meat column, demonstrating that bacteria do migrate vertically downward into the purge of a beef combo. These observations strongly indicate the potential of using a purge sampling method to determine the overall bacterial constituency of a beef combo.

IMPACT/TECH TRANSFER B: Successful validation of this method would have wide impact on the production of ground beef. This study has just been completed. A manuscript has been written and is in peer review. A local ground beef producer has expressed interest in this method and has agreed to allow MARC personnel to conduct validation trials in their plant.

OBJECTIVE C: Develop a rapid microbial ATP bioluminescence assay for determining the microbial level of raw beef combos from a purge sample.

PROGRESS C: The purge from beef combos has been determined to be an effective and representative method for sampling the microbial content of raw beef combos (See Objective D, above).

Purge was collected from vacuum packaged beef and inoculated with fecal bacteria at varying levels. The inoculated purge was assayed for bacteria using both the 24-hr culture and a 1-hr modified rapid microbial ATP bioluminescence method (mR-mATP). Data from this experiment indicated a strong correlation (r = 0.87, n = 102) between the total aerobic bacteria counts derived culturally from the purge samples of raw beef and the total bacterial ATP present in the purge samples. These observations strongly indicate the potential of using this rapid assay on purge samples from raw beef combos to determine the overall bacterial constituency.

IMPACT/TECH TRANSFER C: Field validation is required before the impact of this methodology can be assessed. A local ground beef producer has expressed interest in this method and has agreed to allow MARC personnel to include this method in the validation trial previously mentioned (see Impact/Tech Transfer B).

PUBLICATIONS:

Dorsa, W. J., G. R. Siragusa, C. N. Cutter, E. D. Berry, and M. Koohmaraie. 1996. Recovery of *Salmonella typhimurium* from beef carcass tissue; sponge vs. excision (Study #3). Summary report to the U.S. Food Safety and Inspection Service, "Mega-Reg," March 19.

Dorsa, W. J., G. R. Siragusa, C. N. Cutter, and M. Koohmaraie. 1995. Sampling of beef carcasses at various process point; sponge vs. excision (Study #1). Summary report to the U.S. Food Safety and Inspection Service, 'Mega-Reg," December 29.

Dorsa, W. J., G.R. Siragusa, C. N. Cutter, and M. Koohmaraie. 1996. Recovery of *E. coli* O157:H7 from beef carcass tissue; sponge vs. excision (Study #2). Summary report to the U.S. Food Safety and Inspection Service, "Mega-Reg," January 29.

Dorsa, W. J., G. R. Siragusa, C. N. Cutter, and M. Koohmaraie. 1996. In-plant sampling of beef carcasses; sponge vs. excision (Study #4). Summary report to the U.S. Food Safety and Inspection Service, "Mega-Reg," May 30.

Siragusa, G. R., W. J. Dorsa, C. N. Cutter, L. J. Perino, and M. Koohmaraie. 1996. Use of a newly developed rapid microbial ATP bioluminescence assay to detect microbial contamination on poultry carcasses. *J. Chemilumin. Biolumin.* (In press).

MICROBIAL SAFETY CRITERIA FOR FOODS CONTACTING REUSE WATER IN FOOD PLANTS

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FSIS Number: **I-94–9**
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OBJECTIVE A: Investigate new methods to determine food product safety, water safety, and sanitation quality.

PROGRESS A: F^+RNA coliphage. Investigations were completed to determine the utility of male-specific coliphage as an indicator of water quality. Over 400 samples were split and tested. They included sponge swabs from animal hauling trailer floors, dressed carcasses; fecal material; water from the scald tank, dehairers, gambrel table, polishers; and water from each stage of the water treatment. Numbers of phage plaque forming units per g or mL were usually 1.5 to 2.5 $\log_{10}$ lower than coliform or *E. coli* counts regardless of treatment, including untreated samples. Correlations between virus and bacterial indicators were higher in water than with solid samples. Coliphage is reported to be more chlorine resistant than bacteria; therefore, coliphage may be a more rigorous biomarker for the safety of treated water.

IMPACT/TECH TRANSFER A: Although the relationship between indicator microorganisms and the presence of a pathogen remains contentious, they may be used successfully in the application of HACCP in the food processing environment.

OBJECTIVE B: Determine the potential of reconditioned pork processing plant water to support the growth and/or survival of various Gram negative and Gram positive bacteria.

PROGRESS B: Gram positive bacteria. The Gram-positive pathogens *Staphylococcus aureus* and *Listeria monocytogenes* and the indicator bacterium *Streptococcus faecium* were added to chlorinated and non-chlorinated recondition water from the processing plant's water treatment facility; their survival/growth were then compared to that in potable water obtained from the local township. In contrast to our observations for Gram negative bacteria, the Gram positive bacteria, when added to the waters grew only to a limited extend in the non-chlorinated water which was filtered through a 0.45 mm pore size filter to remove bacterial cells, but generally survived for extended periods, especially at low temperatures. The presence of chlorine, either in potable or chlorinated

reconditioned waters, causes an immediate and rapid decline in viable count of the three bacteria studied.

Gram negative bacteria. The Gram negative pathogens *Salmonella* and *Vibrio cholerae* were inoculated into filter-sterilized (0.45 mm pore size) non-chlorinated reconditioned water and incubated at various temperatures from 5 to 42 °C. Viable counts were done at intervals. *Salmonella* grew well between 17 °C and 37 °C; there was limited growth at 12.4 °C, and survival at the upper temperature of 42.4 °C. *Vibrio cholerae* grew between 10.8 °C and 34.8 °C, with some survival at 39.1 °C. The amount of growth of both pathogens in this reconditioned water correlated with the coliform growth response of the water samples. Both pathogens declined in viable count in either potable or chlorinated reconditioned water.

Survival/growth studies are currently in progress with other Gram-negative pathogens including *Shigella* spp., *Escherichia coli* O157:H7, *V. parahaemolyticus*, *V. vulnificus*, and *Pseudomonas aeruginosa*. Preliminary results indicate that *Shigella* spp. can not grow in reconditioned water while the others grow readily in proportion to the coliform growth response of the water. As with the observations for *Salmonella* and *V. cholerae*, growth is temperature dependent.

IMPACT/TECH TRANSFER B: Our studies with both Gram-positive and Gram-negative bacteria support the observation that residual chlorine levels must be maintained in reconditioned water to inhibit the growth of pathogens which might recontaminate this water after it is purified in their water treatment facility.

OBJECTIVE C: Determine the fate of *Salmonella* on pork head meat treated with alkali.

PROGRESS C: The effectiveness of alkaline washing solutions on reducing the number of *Salmonella typhimurium* on artificially contaminated pork head meat was evaluated in a laboratory setting. The variables studied included solutions of trisodium phosphate (TSP), NaOH, and KOH at different concentrations (pH $\geq$ 12.0), time and temperature of treatment, and treatment method (rinse *vs.* 'tumbling'). TSP treatment yielded a greater reduction of *S. typhimurium* than either NaOH or KOH. A 2.75 $\log_{10}$ reduction of *Salmonella* was observed when 64 mM concentration of TSP (equivalent to a 10% solution) was evaluated at 25 °C for 15 min using a tumbling procedure. Reduction in the number of *Salmonella* by TSP can only be partially described as a pH effect since TSP under similar conditions gave a greater reduction than either NaOH or KOH. Based on these observations, treatment of pork head meat with TSP should reduce the generally encountered *Salmonella* on this meat to undetectable levels.

These findings are being extended to a pilot plant setting within an actual meat processing plant. Treatment of naturally contaminated pork head meat with a 10% TSP solution at *ca* 16 °C results in a 2–3 $\log_{10}$ reduction in both the coliform and aerobic plate count.

IMPACT/TECH TRANSFER C: This research is being performed under a MOU with Hatfield, Inc., at their Hatfield, PA establishment. Supplementary funding was provided by Hatfield, Inc. and the National Pork Producers Council. FSIS has reviewed the proposed protocol under FSIS Directive 10,700.1. It is expected that this research and completed technology transfer to FSIS will occur by September 1997.

OBJECTIVE D: Determine the efficacy of washing and sanitizing hog hauling trailers on the elimination/reduction of *Salmonella* and *E. coli*.

PROGRESS D: The floors of 32 hog hauling trailers were sampled after removal of the bedding material. They were then washed and sanitized according to a standard procedure. *Salmonella* and *E. coli* counts were done before and after the procedure. In most instances, the level of *Salmonella* were reduced to undetectable levels by the procedure, while the *E. coli* count was reduced by about 2 to 3 $\log_{10}$. Analysis of the bedding material showed a consistent presence of *Salmonella*. Season of the year and length of time the hogs were in the trailer had no influence on the incidence of *Salmonella*. These results indicated that washing and sanitizing hog hauling trailers between runs should eliminate *Salmonella* and thus contribute to breaking the transmission cycle for this pathogen.

IMPACT/TECH TRANSFER D: Hatfield, Inc. requested this research under the terms of a MOU. Research was supported in part by Hatfield, Inc. The results provided to the company the scientific basis for washing hauling trailers after each trip. In addition, the information was shared with the National Pork Producers Council and the USDA Technical Analysis Group on Food Transportation.

OBJECTIVE E: Compare and evaluate techniques for determining the bacteriological quality of hog carcass surfaces.

PROGRESS E: Swab and excision methods were compared to determine the levels of *E. coli* on hog carcass surfaces. Working with a small local hog slaughter facility, swab and excision samples from 105 hog carcasses were obtained 24 hr post slaughter. Excision samples were taken from the ham, belly, and jowl; swab samples were taken either at one site (belly) or at three sites with the same swab (ham, belly, and jowl). Swab samples were processed either immediately or after 24 hrs in the cold (to simulate shipping of the samples from sampling point to the laboratory). *E. coli* counts were done using *E. coli* Petri film™ (24 hrs at 37 °C). The data were analyzed by analysis of variance either using as counts or as incidence (counts of $\leq$ were treated as zero). The results indicate that a) excision gave the highest recovery (696/cm^2) compared to either single or multiple site swabs; b) for the multiple site swabs, simulated shipping caused a small decrease in count; and c) for the single site swab, recovery was the same during simulated sample shipping. These results define sampling protocols which will permit optimum recovery of *E. coli* from hog carcass surfaces.

IMPACT/TECH TRANSFER E: This urgent request for research by FSIS was carried out and the data was immediately used by the Agency to support provisions of the HACCP/Pathogen Reduction Regulation. The ARS research provided the scientific basis for the carcass sampling protocol for the determination of *E. coli* on market pig carcasses.

OBJECTIVE F: Develop approaches for detachment of pathogens from meat surfaces.

PROGRESS F: A protocol was developed to study the binding of the bacterial cell surfaces utilizing the BIAcore biosensor. Whole cells of *E. coli* O157:H7 were directly immobilized to the sensor surface. The binding property of the cell surfaces was studied with an antibody against *E. coli* O157:H7. Results demonstrated that the bacterial cell surfaces retained their binding ability in repeated experiments. The binding and dissociation of the antibody with the cell surface was reversible. The binding kinetics of the antibody against the bacterial cell surfaces were determined. With the conditions used, the dissociation and association rates were 10^{-5} and 10^{3}, respectively with a binding (affinity, K) constant of 10^{7}.

IMPACT/TECH TRANSFER F: This procedure shows for the first time the feasibility of using whole cells as capturing ligands. The reversibility of binding and dissociation will be useful in the study of bacterial binding with extracellular matrix components. Results derived from this study can expedite the testing of intervention/detachment schemes.

PUBLICATIONS:

Miller, A. J., J. E. Call, and B. S. Eblen. 1996. Growth and survival of *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Staphylococcus aureus* in brine and propylene glycol chiller conditions. Proceedings of the 42nd International Congress of Meat Science and Technology, Lillehammer, Norway. (In press).

Palumbo, S. A., J. Call, B. Huynh, and J. Fanelli. 1996. Survival and growth potential of *Aeromonas hydrophila* in reconditioned pork-processing plant water. *J. Food Prot.* (In press).

Palumbo, S. A., B. S. Eblen, A. J. Miller, and J. G. Phillips. 1996. Comparison of techniques to evaluate the bacteriological quality of pig carcass surfaces. Proceedings of the 42nd International Congress of Meat Science and Technology, Lillehammer, Norway. (In press).

Rajkowski, K. T., S. A. Palumbo, and A. J. Miller. 1996. Washing/sanitizing of pig trailers to reduce the incidence of *Salmonella*. Proceedings of the 42nd International Congress of Meat Science and Technology, Lillehammer, Norway. (In press).

Rajkowski, K. T., E. W. Rice, B. Huynh, and J. Patsy. 1996. Growth of *Salmonella* spp. and *Vibrio cholerae* in reconditioned wastewater. *J. Food Prot.* **59**:577–581.

SPECTRAL RADIOMETRY AS AN ON-LINE INSPECTION TOOL

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FSIS Number: **I-91–6**
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OBJECTIVE A: Develop an automated, real-time system for on-line detection of unwholesome poultry carcasses, as defined by the Food Safety Inspection Service (FSIS), in slaughter plants.

PROGRESS A: A visible/near-infrared (Vis/NIR) spectrophotometer system was tested for on-line classification of wholesome and unwholesome carcasses. Vis/NIR spectra of wholesome and unwholesome poultry carcasses on a moving shackle were obtained using a spectrophotometer system, either under room light or in a dark environment. The unwholesomeness included cadaver, septicemia, airsacculitis, bruise, ascites, and tumor. The shackle speed was set either at 60 or 90 birds/min and the distance between the probe and the carcass was set within 2 to 5 cm. The scanning time was 0.32 seconds per carcass.

Neural network models and PCA/MD (Principal Component Analysis/Mahalanobis Distance) classifiers were developed on a calibration set and validated on a test set, which did not include any samples from the calibration set. All models showed that the Vis/NIR spectrophotometer system could classify the wholesome or unwholesome carcasses with accuracies higher than 94%. The best results were obtained with the 90 birds/min shackle speed and sensing in the dark. The accuracies were 96.0% for classifying wholesome carcasses and 98.9% for unwholesome carcasses. The results also showed that the room light intensity was too low, compared to the spectrophotometer system's tungsten halogen light source, to make any impact on the measurement. All these results indicated that the Vis/NIR spectrophotometer system can be used for on-line, real-time classification of wholesome and unwholesome carcasses with very high accuracy.

A multiple spectral imaging technique also was tested for the separation of wholesome and unwholesome carcasses. The system acquires spectral images from the chicken on a moving shackle in real-time and processes these spectral data for classification. The spectral images of 540 nm and 700 nm wavelengths were useful for separating unwholesome carcasses (including ascites, airsacculitis, bruise, cadaver, leukosis, septicemia, and tumor) from the wholesome carcasses based on spectral image pixel intensity and the intensity distribution of Fourier power spectrum. The best neural network classifier was obtained when spectral image pixel intensity of 540 nm and 700 nm wavelengths were combined and used as inputs. The accuracy of validation was 93.3%.

A pilot-scale instrumental inspection system for poultry carcasses was assembled and tested. The system includes a Vis/NIR spectrophotometer system for scanning the breast of the bird and a spectral imaging system which consists of 2 cameras with 540 and 700 nm filters for imaging the front of the bird and 2 additional cameras with same filters for imaging the back of the bird. This pilot-scale system can operate 62 birds moving at a speed up to 100 birds per min. Presently, both of the Vis/NIR system and the spectral imaging system can operate up to 100 birds per minute. As of this date, three high speed personal computers (Pentium, one 166 MHz and two 200 MHz machines) and one high speed spectrophotometer (10 times faster than the current system) are ordered and will be implemented in the near future. As a result of the higher processing speed equipment, the speed of instrumental inspection could be greatly increased.

IMPACT/TECH TRANSFER A: The development of our on-line instrumentation inspection system for poultry carcasses was presented to the National Broiler Council's Processing Committee at Atlanta, GA. A description of the on-line instrumentation inspection system was presented to FSIS's Technology Transfer and Coordination Staff. The application of the instrumentation on-line poultry inspection system also was presented to the Director of Chicken Processing and the Plant Manager of Wampler Foods Moorefield Poultry Slaughter Plant. Utech Inc., Bethesda, MD, has expressed its interest in entering a CRADA with ARS on commercializing the inspection system.

PUBLICATIONS:

Chen, Y. R., R. W. Huffman, and B. Park. 1996. Changes in the visible/NIR spectra of chicken carcasses in storage. *J. Food Process Eng.* **19**(2):121–134.

Chen, Y. R., R. W. Huffman, B. Park, and M. Nguyen. 1996. A transportable spectrophotometer system for on-line classification of poultry carcasses. *J. Appl. Spectrosc.* **50**(7):910–916.

Chen, Y. R., B. Park, M. Nguyen, and R. W. Huffman. 1996. Instrumental system for on-line inspection of poultry carcasses, pp 121–129, SPIE Vol 2786. Proceedings of the International Symposium on Lasers, Optics, and Vision for Productivity in Manufacturing, Besançon, France.

Park, B. and Y. R. Chen. 1996. Multispectral image co-occurrence matrix analysis for poultry carcass inspection. Transactions of the ASAE **39**: (In press).

Park, B., Y. R. Chen, and R. W. Huffman. 1996. Integration of visible/NIR spectroscopy and multispectral imaging for poultry carcass inspection. *J. Food Eng.* (In press).

Park, B., Y. R. Chen, M. Nguyen, and H. Hwang. 1996. Characterizing multispectral images of tumorous, bruised, skin-torn, and wholesome poultry carcasses. Transactions of the ASAE **39**: (In press).

Part III. POST-SLAUGHTER PATHOGEN CONTROL

MICROBIAL MODELING

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OBJECTIVE A: Develop mathematical models to estimate the behavior of foodborne pathogens.

PROGRESS A: The Pathogen Modeling Program, Version 4.0, continues to be distributed to microbiologists in all areas of the food industry. The CRADA with Simusoft, Inc. to commercially develop the next version of the program was terminated and ARS resumed control of software development and distribution. The program was given minor corrections and updates and then made available via the INTERNET through FTP (file transfer protocol). Work was begun to write a completely new software package for the PMP that will operate under the Windows environment, be much simpler and faster in its execution, and provide confidence intervals for all of the predictions.

E. coli O157:H7. Growth, survival and thermal death rates of 16 strains of *E. coli* O157:H7 are being measured to determine the natural variation in identical environments. Lag times had much more variation than the exponential growth rates. Survival times in broths simulating the pH, lactate, nitrite and salt content of fermented meat product ranged from 100 to 800 hrs depending upon the strain. Initial data suggests a twofold range in thermal D-values between the least and most hardy strain.

The cooperative project funded by USDA-OICD and the European Union with the National Food Centre, Dublin, Ireland, determined that the Salami outbreak strain of *E. coli* O157:H7 would only decrease 1.5 to 2 logs during the standard 37 °C fermentation and drying of pepperoni manufacture. Changes in the pH, salt or nitrite levels that would be feasible did not greatly affect the decrease.

In another cooperative project with the National Food Centre, Dublin, Ireland, a visiting scientist from NFC investigated the competition between *E. coli* O157:H7 and the starter cultures or the normal spoilage flora. Experiments were conducted in broth media

simulating a fermented meat product, data were collected, and they are being analyzed. Initial review shows that these microorganisms do reduce the growth of *E. coli* O157:H7.

Predatory bacteria *Bdellovibrio* that prey upon *E. coli* and *Salmonella* species were isolated from soil and sewage samples. The isolates attacked and lysed the prey bacteria with log reductions ranging from 2.5 to 7.9 CFU/mL after 7 hrs incubation. *Bdellovibrio* isolate 45k reduced a population of *E. coli*, dried on stainless steel surfaces, by 3.6 log CFU/mL following 24 hrs of contact time at a 10:1 predator to prey ratio and also was effective in reducing the level of biofilm cells.

Growth characteristics of *Shigella flexneri* observed in foods compared favorably with the growth predicted by the aerobic growth model (previously developed) at temperatures of 19–37 °C, but not at temperatures below 19 °C. Additional data were collected for growth in BHI media at 10–19 °C in order to expand the growth model. The new data were combined with the previous data and submitted for statistical analysis. Four response surface models with terms for temperature, pH, sodium chloride, and sodium nitrite were obtained and are being evaluated.

Listeria monocytogenes. The lag phase of a microorganism is affected by the previous history and conditions, as-well-as it's present environment. Previous modeling began with cells of *Listeria monocytogenes* grown in a favorable medium to the late exponential growth phase. Therefore, *L. monocytogenes* cells were grown at selected temperatures from 4 to 28 °C and then transferred to a fresh medium at various temperatures. The lag times were shorter when transferred to the higher temperature broths, but within a temperature, the shortest lag time was for cells with the least temperature change. The lag time for cells transferred from temperatures of 28 and 37 °C to 4 and 8 °C were longer than cells initially grown at temperatures < 28 °C. The shortest lag times were for cells in the exponential growth phase, and the longest time was for cells that were starved or desiccated. After the lag phase was completed, the exponential growth rates were not affected by the prior history or state of the cells (see "Growth and Toxin Production of Harmful Psychotrophs," page 89, by Miller, Palumbo, Bhaduri, and Fratamico for additional information).

Growth models for *Listeria monocytogenes* were expanded. Data compiled over the past several years were incorporated into the previous data set for *L. monocytogenes* and used to expand the model for this microorganism. While the final analysis on approximately 1500 growth curves is being completed, it appears that a set of two quadratic models, one for temperature/pH/NaCl/$NaNO_2$, and the other for temperature/pH/water activity/ $NaNO_2$, for aerobic and anaerobic growth will be available shortly to update the Pathogen Modeling Program.

Growth kinetics, cell morphology and cellular proteins were determined for *L. monocytogenes* Scott A. grown under stress. Studies were conducted using BHI broth (pH 6.0) at 19, 28, 37, and 42 °C. Control cultures contained 0.5% NaCl; test cultures contained NaCl (4.5, 6.0 or 7.5%) or EDTA (0.1, 0.2 or 0.3 mM). Addition of NaCl or

EDTA increased both lag and generation times. Addition of 0.3 mM EDTA prevented growth at 37 and 42 °C. Stationary phase cells were examined by scanning electron microscopy and cellular proteins were examined by one-dimensional SDS polyacrylamide gel electrophoresis. Cells grown in control media at 19 and 28 °C were short rods with flagella. Growth under increasingly stressful conditions (higher temperatures, higher NaCl or EDTA levels) resulted in cell elongation and loss of flagella. Differences in total cellular protein patterns were mainly due to temperature. Cell length did not correlate with growth kinetics. All growth conditions studied significantly affected the properties of *L. monocytogenes* cells.

An important issue for growth modeling is whether an interaction (competition) between *Listeria monocytogenes* and spoilage microorganisms exists. The effect of temperature, pH, and sodium chloride content on the interaction of *L. monocytogenes* and *P. fluorescens* was evaluated using mono- and co-cultures. The spoilage organism's effect on the pathogen varied depending on the environmental conditions; suppressing pathogen growth in some instances and promoting it in others. While there was some indication that relative growth rate is one of the determinants determining the impact of *P. fluorescens*, the effect was not nearly as definitive as earlier studies with *Carnobacterium piscicola*. Overall, the data for the two spoilage organisms indicate that modeling the interaction of species is going to be very difficult, particularly when biovariability among strains also is considered.

The model for the non-thermal inactivation of *Listeria monocytogenes* was expanded. The analysis of the combined data sets for the inactivation of *L. monocytogenes* was completed and a series of five variable (temperature, pH, acid concentration, sodium nitrite, and sodium chloride/water activity) response surface models were generated for the aerobic data set. Two quadratic models, one for sodium chloride content and one for water activity, were found to effectively model the inactivation of *L. monocytogenes* in acid environments.

IMPACT/TECH TRANSFER A: Copies of the pathogen modeling program have been sent to over 700 microbiologists in 32 countries in industry, academia, and government. The Internet availability will increase access and make the planned upgrades of the software easier to do. This software has become a standard tool for food microbiologists. The improvements will change it from being able to calculate single conditions to modeling an entire process. This will greatly facilitate its use in designing HACCP programs and in eventually conducting risk assessments.

PUBLICATIONS:

Fratamico, P. M and Cooke, P. H. 1996. Isolation of *Bdellobibrios* that prey on *Escherichia coli* O157:H7 and *Salmonella* species and application for removal of prey from stainless steel surfaces. *J. Food Saf.* **16**:161–173.

INTERVENTIONS AND RISK MODELING TO IMPROVE THE MICROBIOLOGICAL SAFETY OF POULTRY PRODUCTS

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OBJECTIVE A: Develop a computer software application that contains mathematical models that predict the number of *Salmonella* on poultry products as they move from the farm to the consumer.

PROGRESS A: Research at this worksite is aimed at developing mathematical models that predict the number of *Salmonella* on poultry products under a variety of environmental conditions encountered in the farm to table continuum. A problem with developing predictive models for *Salmonella* is which isolate(s) to use as there are over 2,000 serotypes. Although effects of species on thermal inactivation kinetics of *Salmonella* has been well-investigated, less is known about effects of species on growth characteristics of *Salmonella*. Consequently, isolates of *Salmonella* from poultry operations were surveyed for their growth characteristics in laboratory medium for the purpose of identifying isolates suitable for modeling experiments. In Experiment 1, fourteen poultry and two ATCC isolates of *Salmonella* were grown at 40 °C in brain heart infusion broth (BHI) adjusted to pH 6. Lag time (LT; $P = 0.07$) and maximum population density (MPD; $P = 0.0005$) differed between isolates, whereas growth rate (GR) did not. Most isolates exhibited similar growth characteristics with LT ranging from 1.3 to 1.9 hr and MPD ranging from 9.2 to 10.3 $\log_{10}$ colony forming units (CFU) per mL. In experiment 2, three poultry and one ATCC isolate were grown to stationary phase at 37 °C and then switched to 5, 22, or 37 °C for 24 hr before measurement of growth characteristics at 37 °C in BHI adjusted to pH 6. Preincubation temperature did not alter or interact with isolate to alter growth characteristics. The isolate had a slight effect ($P = 0.06$) on MPD but did not alter LT or GR. Overall, growth characteristics in BHI were not markedly different between isolates of *Salmonella* indicating that all the isolates examined were suitable for modeling experiments.

IMPACT/TECH TRANSFER A: Results of this isolate survey will allow us to proceed to the next stage of the project which is to develop predictive models for estimating changes in *Salmonella* numbers on poultry as it moves through the farm to table chain. It is envisioned that the predictive models will be incorporated into quantitative risk assessment models and used by FSIS for estimating the risk of acquiring salmonellosis from consumption of poultry products produced by different farm to table scenarios.

OBJECTIVE B: Determine the utility of an automated DNA fingerprinting system, the DuPont RiboPrinterOE, for identifying isolates of *Salmonella* from poultry operations.

PROGRESS B: A study was conducted to determine the frequency with which the RiboPrinterOE, an automated DNA fingerprinting system, identifies isolates of *Salmonella* at the serovar level. Eighty-three isolates of *Salmonella* from poultry operations, primarily broiler growout houses, were subjected to DNA fingerprint analysis by the RiboPrinterOE. The RiboPrinterOE positively identified 42.2% of the isolates at the serovar level. The other isolates were not identified at the serovar level because their RiboPrinter patterns did not match a RiboPrinter pattern in the RiboPrinterOE database (8.4%), their RiboPrinterOE pattern was too similar to other serovars for a positive identification to be made (36.1%), or their DNA was not successfully processed by the RiboPrinterOE (14.4%).

IMPACT/TECH TRANSFER B: Results of this study indicate that the RiboPrinterOE is limited in its ability to identify *Salmonella* at the serovar level and therefore, would need to be used in combination with classical serotyping in an epidemiological study aimed at identifying critical control points in the farm to table continuum for *Salmonella* contamination of poultry products.

GROWTH AND TOXIN PRODUCTION OF HARMFUL PSYCHROTROPHS

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OBJECTIVE A: Study the mechanism that allows foodborne pathogens to adapt to psychrotrophic growth and determine the impact of this adaptation on the organism's pathogenicity.

PROGRESS A: *E. coli* O157:H7. Four strains of hemorrhagic *E. coli* (1558, 1772, O157:NM1, and O157:NM3) were added to either sterile or pasteurized 2% milk (low or high background microflora levels), incubated at 5, 8, 12, and 15 °C, and the number of *E. coli* and background microflora were determined at intervals by plating on appropriate media. When the *E. coli* increased in numbers, samples were taken for the verotoxin assay using a standard tissue culture assay. In the absence of background microflora, strain 1772 grew at 8 °C, but did not produce verotoxin; the three other strains grew at 12 °C, with better growth in the absence of background flora. Only limited amounts of verotoxin were formed at 15 °C, the highest abuse temperature tested. Under conditions which did not favor growth (too low a temperature or too high a background flora count), the four strains remained viable. Milk itself did not appear to be a very adequate substrate to support verotoxin production. When the four strains were inoculated individually into sterile milk and incubated at 37 °C, the optimum temperature of growth for these bacteria, only very limited amounts of verotoxin were formed compared to incubation in BHI culture broth.

Listeria monocytogenes. *Listeria monocytogenes* inoculum growth temperature and cell phase history were studied to determine effects on the lag phase duration (LPD) in brain heart infusion (BHI) broth. Initially, inocula were grown at 5, 12, 15, 19, 28, or 37 °C to $\log_{10}$ 9 CFU/mL. Flasks were inoculated and maintained at 5, 12, 19, or 28 °C, with periodic sampling and enumeration on BHI agar. The strain also was grown to 5, 7, or 9 $\log_{10}$ CFU, or to stationary phase, at 5 and 37 °C. Inocula were then transferred to 5 °C and enumerated. All growth curves were fitted using the Gompertz equation. For the first series, final incubation temperature was the major factor influencing the LPD, although inocula that were temperature shifted downward exhibited a LPD that increased in proportion to the temperature differential. Generation time was unaffected by temperature shifts and appeared to be a function of final incubation temperature. In the

second series, LPD of transferred cells decreased as the inocula progressed further into log phase, while stationary phase inocula produced an extended lag phase.

IMPACT/TECH TRANSFER A: The observation that inoculum history can influence the LPD of *L. monocytogenes* provides critical information for the design of food preservation systems that maintain product safety. In *E. coli*, study of results indicated that hemorrhagic *E. coli* can grow but not produce verotoxin in milk held at mild abuse temperatures.

OBJECTIVE B: Development of selective enrichment, isolation and identification of plasmid-bearing virulent strains of *Yersinia enterocolitica* from ground pork.

PROGRESS B: A 72 hr at 12 °C enrichment of virulent strains of *Yersinia enterocolitica* (YEP^+) from ground pork was developed, along with a novel isolation and confirmation method. Taken together this new procedure reduces isolation and confirmation time from 3–4 weeks down to 6 days. Modified trypticase soy broth was used to nonselectively enrich samples for 24 hrs and then Irgasan was added (4 mg/mL final concentration) for selective enrichment for an additional 48 hrs. Selectively enriched cultures were diluted and plated at 28 °C on BHA (for quantification) and MacConkey's agar (for presumptive isolation). Presumptive colonies were streaked on Congo red agarose medium (CR-BHO) and incubated at 37 °C for 24 hrs. YEP^+ colonies were identified using characteristic colony morphology (red pinpoint colonies with an occasional white border around the red pinpoint colonies). Virulence of the CR^+ colonies were further confirmed using multiplex PCR for chromosomal *ail* gene (attachment-invasion locus) and *virF* gene (transcriptional activator for the expression of the virulence plasmid-encoded outer membrane protein *yop* 51). The presence of the *ail* gene specifically differentiates *Y. enterocolitica* from *Y. pseudotuberculosis* and the presence of *virF* gene detects the virulence plasmid. As-low-as 9 CFU of YEP^+ per g of spiked ground pork can be detected. This technique has been successfully applied to other virulent serotypes including 0:8, 0:5 0:27, 0:13 and 0:TAC from ground pork. The procedure was tested on unspiked pork meat samples including ground pork (twenty samples), head meat (ten samples), and tongue (thirty samples). Only tongue was found to contain YEP^+ strains. All isolates from tongue were serotype O:3. YEP^+ strains isolated from both artificially contaminated ground pork and naturally contaminated tongue were found to express plasmid-associated virulence characteristics including colonial morphology, CV binding, low calcium response, CR uptake, hydrophobicity and autoagglutination. The mouse virulence test of the YEP^+ strains isolated from spiked ground pork and naturally contaminated tongue was positive.

IMPACT/TECH TRANSFER B: The technology was transferred to the FSIS Microbiology Division through a hands-on demonstration in Beltsville and by providing a detailed written description.

OBJECTIVE C: Develop a sampling method for the determination of *E. coli* O157:H7 using beef carcass water washes.

PROGRESS C: Studies were continued on an *E. coli* O157:H7 carcass sampling method using beef carcass wash water. The bacteria in the wash water were collected on membrane filters, removed from the filters and the material was tested by the following methods: by an ELISA technique with colorimetric detection, by multiplex PCR followed by agarose gel electrophoresis and ethidium bromide staining, and by a direct epifluorescence technique in which brightly fluorescent *E. coli* O157:H7 are visualized microscopically.

IMPACT/TECH TRANSFER C: These techniques will allow the ready and rapid detection of *E. coli* O157:H7 on whole animal carcasses by examining wash water from the carcasses.

OBJECTIVE D: Assess the utilization of biomolecular interaction analysis for the rapid detection of *E. coli* O157:H7.

PROGRESS D: Studies were continued on the use of the BIAcore biosensor for detection of *E. coli* O157:H7. Initial studies included using monoclonal and polyclonal antibodies against *E. coli* O157:H7 as ligands on the sensor chip surface followed by injection of the bacteria. This was followed by an injection of monoclonal or polyclonal antibodies in order to enhance the resonance signal. Studies are in progress to detect the pathogen using an inhibition assay. Bacteria are allowed to react with anti-*E. coli* O157:H7 antibodies in solution and the material remaining following centrifugation is injected over a Protein G surface on the sensor chip. Since Protein G binds the Fc portion of IgG, the resonance signal drops as the number of *E. coli* in the sample increases.

IMPACT/TECH TRANSFER D: Use of the BIAcore biosensor will permit the real time detection of *E. coli* O157:H7. The BIAcore detection sensitivity is currently 10^7–10^8 CFU/mL.

OBJECTIVE E: Develop a laboratory control strain of E. coli O157:H7.

PROGRESS E: The *Aequorea victoria* green fluorescent protein gene on plasmid vector pGFP was introduced into strains of *Escherichia coli* O157:H7. The recombinant strains were indistinguishable from their parent strains in biochemical and immunological tests and in a multiplex PCR assay. When grown at 37 or 42 °C both with and without antibiotic selection, the recombinant strains maintained pGFP and expressed the green fluorescent protein. The recombinants emitted bright green fluorescence when excited with UV light and the addition of any other proteins, substrates or cofactors was not required.

IMPACT/TECH TRANSFER E: These *E. coli* O157:H7 strains, containing a readily identifiable and stable marker, could be useful as positive controls in microbial assays as-

well-as in studies monitoring bacterial survival and the behavior of the pathogen in a food processing environment.

OBJECTIVE F: Determine conditions that affect the expression of virulence factors associated with the attachment of enteric pathogens to tissue.

PROGRESS F: *E. coli* O157:H7 expresses "curli" type fimbriae under certain conditions. *E. coli* and other bacterial genera were screened for the presence of the *crl* and *csgA* genes by PCR. Expression of these fimbriae is being examined by RT-PCR and by scanning electron microscopy. An assay also is being developed to correlate expression of curli fimbriae to the attachment to intestinal cells.

IMPACT/TECH TRANSFER F: None.

PUBLICATIONS:

Bhaduri, S. 1996. Presence of virulence plasmid in *Yersinia enterocolitica* after its expression at 37 °C. *Rapid Methods Autom. Microbiol.* (In press).

Deng, M. Y. and P. M. Fratamico. 1996. A multiplex PCR for rapid identification of Shiga-like toxin-producing *Escherichia coli* O157:H7 isolated from foods. *J. Food Prot.* **59**:570–576.

Fratamico, P. M., S. K. Sackitey, and M. Weidmann. 1996. Assay for enterohemorrhagic *Escherichia coli* O157:H7 by the polymerase chain reaction. U.S. Patent Application Number 08/357,791. (Granted in March).

Fratamico, P. M., F. J. Schultz, R. C. Benedict, R. L. Buchanan, and P. H. Cooke. 1996. Factors influencing attachment of *Escherichia coli* O157:H7 to beef tissues and removal using selected sanitizing rinses. *J. Food Prot.* **59**:453–459.

HEAT RESISTANT *CLOSTRIDIUM BOTULINUM* SPORES/IMPACT OF CHANGING TECHNOLOGIES

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OBJECTIVE A: Define the heat treatment required to achieve a specified lethality for *Escherichia coli* O157:H7 in 90% lean beef and chicken, and also assess the change in heat resistance of *E. coli* O157:H7 in beef during refrigerated and frozen storage.

PROGRESS A: Thermal inactivation of a four-strain mixture of *E. coli* 0157:H7 was determined in 90% lean ground beef, and lean ground chicken. D-values (time to inactivate 90% of the population) were calculated from the straight portion of the survival curves by plotting log of survival counts vs. their corresponding heating times. Also, regression lines were fitted to experimental data points that contributed to tailing or shouldering by a survival equation (model) developed by Dr. Whiting at ERRC using the Gauss-Newton curve fitting program (ABACUS Software Program, ERRC, USDA, Philadelphia, PA) and two D-values were calculated, one for the major population and another for a subpopulation. The Z-values were estimated by computing the linear regression of mean $\log_{10}$ D-values vs. their corresponding heating temperatures. D-values, determined by linear regression, in beef were 21.13, 4.95, 3.17, 0.93 and 0.39 min, respectively (Z = 6.0 °C). Using a survival model for non-linear survival curves, D-values in beef ranged from 20.45 min (D_1, and there was no D_2) at 55 °C, to 0.16 min (D_1) and 1.45 min (D_2) at 65 °C. When *E. coli* O157:H7 four-strain cocktail was heated in chicken, D-values calculated by both approaches were consistently less at all temperatures. This shows that if lean ground beef is used to validate the safety of a process for *E. coli* O157:H7, that process also will be safe for lean chicken. The heat resistance of *E. coli* O157:H7 was substantially increased after refrigerated or frozen storage of inoculated beef for 48 hrs.

IMPACT/TECH TRANSFER A: Thermal death time values from this study will assist food processors in designing acceptance limits on critical control points that ensure safety against *E. coli* O157:H7 in cooked beef and chicken.

OBJECTIVE B: Quantify the inactivation of *E. coli* O157:H7 and spoilage aerobic microflora in ground beef patties cooked in a skillet.

PROGRESS B: Ground beef patties inoculated with a five-strain mixture of *E. coli* O157:H7 were cooked on a Farberware skillet set at a temperature of 275 °F (137 °C). When surviving *E. coli* O157:H7 or spoilage microflora were plotted vs. end-point temperature of the hamburger, the r^2 values were 0.94 for *E. coli* O157:H7 and 0.96 for spoilage microflora. Ground beef patties heated to an internal temperature end-point of 155 °F (68.3 °C) resulted in 4 $\log_{10}$ CFU/g reductions of the organism. Normal spoilage microflora in a hamburger died almost at the same rate as did the inoculated *E. coli* O157:H7 strains in the hamburger.

IMPACT/TECH TRANSFER B: This investigation conducted under conditions simulating those that occur in the retail food industry should ensure safety against *E. coli* O157:H7 in ground beef patties. The finding that the spoilage microflora die almost the same as the *E. coli* O157:H7 would provide a simple way, without introducting pathogens in a retail food operation, to develop a process authority to validate the safety of a cooking process in the retail food operation.

OBJECTIVE C: Determine the effect of heat shocking *E. coli* O157:H7 inoculated in meat on induced thermotolerance in these cells and the persistence of the thermotolerance after heat shock. Also, define the time/temperature necessary for complete killing of bacterial cells.

PROGRESS C: The effect of prior heat shock on heat resistance of *E. coli* O157:H7 in ground beef was investigated. When ground beef samples inoculated with a cell culture suspension of a four strain cocktail of *E. coli* O157:H7 were subjected to sublethal heating at 46 °C for 15 and 30 min, and then cooked to a final internal temperature of 60 °C, heat shocking allowed the organism to survive longer than nonheat shocked cells; but, 15 or 30 min heat shock did not show a significant difference. D-values at 60 °C increased 1.5-fold after heat shocking. The induction of thermotolerance by heat shock was maintained after 24 hrs at 4, 15 or 28 °C. When ground beef samples containing the heat-shocked organisms were heated to a final test temperature of 60 °C for 45 min and stored at 4, 15 or 28 °C for 24 hrs to allow the heated cells to recover in the heating medium, as high as 7 $\log_{10}$ CFU/g were recovered from 28 °C stored samples. However, longer heating (60 min) at 60 °C completely killed the bacterial cells as indicated by no recovery of the organism during storage.

IMPACT/TECH TRANSFER C: Food processors should take into account increased thermotolerance when designing thermal processes for meat products.

OBJECTIVE D: Determine the heat resistance of *Clostridium perfringens* vegetative cells in ground beef and turkey.

PROGRESS D: Heat inactivation of a three-strain mixture of *Clostridium perfringens* vegetative cells was determined in ground beef and turkey. Surviving cells were

recovered on tryptose sulphite cycloserine agar. D-values in beef ranged from 1 min at 62.5 °C to 21.6 min at 55 °C; the values in turkey ranged from 0.98 min at 62.5 °C to 17.5 min at 55 °C. Heating to an internal temperature of 65 °C inactivated approximately 7 $\log_{10}$ CFU/g of bacterial cells as indicated by no recovery of the organism.

IMPACT/TECH TRANSFER D: The findings, addressing FSIS needs, were sent to the FSIS PPID/HACCP Division which used the information to aid with the disposition of products subject to cooling deviations.

OBJECTIVE E: Develop a rapid technique for the specific detection of viable *Listeria monocytogenes* based on reverse transcription-polymerase chain reaction (RT-PCR) technology.

PROGRESS E: A method for the specific detection of viable *L. monocytogenes* was developed based on RT-PCR amplification of a portion of the *iap* mRNA of *L. monocytogenes*. Following a 1 hr enrichment incubation, total RNA was isolated and DNase-treated. A 318 bp fragment of the *iap* mRNA was amplified by RT-PCR and the amplified product detected by Southern hybridization to an internal digoxigenin-labeled probe. The assay is specific for viable cells; production of the *iap* fragment resulted from amplification of *iap* mRNA, not the *iap* gene, since no signal was detected upon amplification of the RNA by conventional PCR using Taq DNA polymerase. The RT-PCR assay was specific for *L. monocytogenes* and demonstrated a level of sensitivity in the range of 5 CFU/mL in pure broth culture.

IMPACT/TECH TRANSFER E: The rapid diagnostic test should provide FSIS the scientific knowledge needed for regulatory decisions and will be of value for analysis of foodborn pathogens in cooked foods.

PUBLICATIONS:

Juneja, V. K., B. S. Marmer, and J. E. Call. 1995. Influence of modified atmosphere packaging on growth of *Clostridium perfringens* in cooked turkey. *J. Food Saf.* **16**:141–150.

Juneja, V. K., B. S. Marmer, J. G. Phillips, and S. A. Palumbo. 1995. Interactive effects of temperature, initial pH, sodium chloride and sodium pyrophosphate on the growth kinetics of *Clostridium perfringens*. *J. Food Prot.* **59**(8):1–7.

OPTIMIZATION OF THE SAFETY, QUALITY, AND SHELF-LIFE OF IRRADIATED POULTRY AND RED MEAT

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OBJECTIVE A: Compare the effectiveness and predictability of food irradiation processing on meats that are not generically related, specifically comparison of the resistance to gamma-radiation of *Salmonella* or *Staphylococcus aureus* in bison (bovine), ostrich (ratite), alligator and caiman (reptile) meats.

PROGRESS A: There is an expanding industry for the marketing of high value meats from animals other than the typical domesticated species, including, but not limited to, bison, ostrich, alligator, and caiman. In this study we compared the gamma-radiation resistance of a mixture of *Salmonella* (*S. dublin, S. enteritidis, S. newport, S. senftenberg,* and *S. typhimurium*) and a mixture of *Staphylococcus aureus* (ATCC 25923 and B124) when present on ground bison, ostrich, alligator, and caiman meats at 5 °C. A minimum of five doses were used to establish the D-values, and the studies were replicated three times. Because the type of meat did not significantly alter the radiation resistance of either *Salmonella* or of *S. aureus*, all of the results for each organism were combined to obtain the following radiation D-values of 0.53 ± 0.02 and 0.37 ± 0.01 kGy for *Salmonella* and *S. aureus*, respectively. The authors conclude that both of these foodborne pathogens can be eliminated from these meats by gamma-radiation doses between 1.5 and 3.0 kGy at 5 °C. These are levels currently approved by the FDA and USDA for the irradiation of poultry.

IMPACT/TECH TRANSFER A: Results provide FDA and FSIS with data to support the concept of the general applicability of radiation resistance data for foodborne pathogens to all meat and poultry products. Results will be provided to the relevant meat processing industries for their possible use in petitions to the FDA. A CRADA is nearing completion with GrayStar, Inc, Mt. Arlington, NJ, to further apply gamma-irradiation for the improvement of agricultural products.

OBJECTIVE B: Evaluate, using modeling techniques, the delay in the onset of microbial spoilage of refrigerated chicken by gamma-irradiation.

PROGRESS B: The multiplication of the indigenous bacterial flora on gamma-irradiated (0, 0.75, 1.5, 2.25, and 3.0 kGy) chicken drumsticks stored at 4 °C within oxygen-permeable packaging for up to 21 days is described and modeled using a modification of the Gompertz equation. Lag phases for the indigenous bacterial flora and the shelf life of the irradiated drumsticks increased uniformly with radiation dose. A dose of 2.25 kGy is predicted to produce an approximate shelf life of 14 days for irradiated drumsticks. The effect of irradiation dose on the microbial population on the irradiated drumsticks was described with a quadratic response-surface model. Though sensory analyses were of secondary importance in this study, the results were in good agreement with the bacteriological results and with those of other investigators. The effect of storage temperature (5, 10, and 20 °C) on the multiplication of bacteria on irradiated (0, 1.25, and 2.5 kGy) mechanically deboned chicken was modeled using quadratic response surface equations. Because of a larger initial population and a much larger ratio of surface area to mass of the mechanically deboned chicken, spoilage occurred in less time. Irradiation of the product increased the lag times and delayed the onset of spoilage.

IMPACT/TECH TRANSFER B: The results are expected to be of value to scientists, regulatory agencies, and poultry processors.

OBJECTIVE C: Determine the effect of low temperature enzyme denaturation on radiation sensitive components of pork during irradiation, and their stability during subsequent cooking and storage.

PROGRESS C: Pork *L. dorsi* muscle tissue was ground and packaged in anaerobic foil packages under nitrogen. One third of the samples were heated to 70 °C to heat denature the proteins prior to irradiation. All samples were then irradiated and another third of the samples were post-irradiation heat treated. Samples were both cooked and stored, with all possible combinations of treatments represented. The only irradiation effect noted was the loss of thiamin; the loss of thiamin was not affected by heating either before or after irradiation. Neither thiamin nor α-tocopherol content changed during storage. Thus irradiation and heating under anoxic conditions did not interact synergistically as has been noted in the presence of oxygen. The only other effect noted was that heat treatments at either 70 °C or 98 °C, caused an increase in the amount of measured α-tocopherol. This was attributed to saponification of the tocopherol esters. The lack of any loss of vitamins during cooking or storage or specifically of riboflavin and α-tocopherol during irradiation was due in part to the exclusion of oxygen from the samples during preparation and subsequent treatment.

IMPACT/TECH TRANSFER C: This study will help the food industry design irradiation processes for lightly processed foods that will maximize nutrient retention.

OBJECTIVE D: Develop a practical method to identify irradiated poultry.

PROGRESS D: A simple, inexpensive, aqueous method is described for screening poultry breast tissue to determine whether it has been irradiated. Formaldehyde was observed to be generated in low ppm amounts, when processed with gamma-radiation at levels between 0–3 kGy at 5 °C. The concentration of formaldehyde detected was no greater than reported to be present in other non-irradiated foods. The formaldehyde is derivatized forming a fluorescent compound that is measured spectrophotofluorometrically. Differences between non-irradiated and irradiated (2 kGy) tissue stored at 4 °C for up to 8 days or frozen for at least 4 weeks are large enough to correctly identify irradiated samples with an accuracy of 92%. The method is not applicable to other poultry parts and to other animal tissue. Approximately 20 breasts can be screened per day per analyst.

IMPACT/TECH TRANSFER D: A low cost, inexpensive method is available for screening purposes by regulatory agencies.

OBJECTIVE E: Develop a simple method of inducing acid tolerance in enterohemorrhagic *E. coli* and use it to characterize the acid tolerance of different isolates.

PROGRESS E: Overnight culturing of enterohemorrhagic *E. coli* in tryptic soy broth with 0 and 10 g of dextrose per liter was found to be an easy means of inducing acid tolerance. When used to characterize eight strains (six O157:H7, one O111:H$^-$, and one reference) by exposing cells (up to 7 hrs at 37 °C) to BHI adjusted to pH 2.5 and 3.0 using HCl, the results indicated that all glucose-grown cells were acid tolerant; whereas, differences in acid tolerance were noted among the strains when grown without glucose. The results suggest that there may be genetic diversity in the acid tolerance response of different strains. The results suggest that stationary phase cells may have two forms of acid tolerance; a *rpo*S-associated form linked to the attainment of stationary phase and an inducible form that is associated with the prior exposure to acidic conditions.

IMPACT/TECH TRANSFER E: This technique provides researchers with a simple means of inducing acid tolerance in pathogenic bacteria so that the potential for acid tolerance to induce increased resistance to other food processing related stresses (e.g., thermal processing, antimicrobials) can be assessed. The results also provide information needed by researchers on potential mechanisms responsible for inducing an acid tolerant state.

OBJECTIVE F: Characterize the effect of pH and pH history on the irradiation resistance of enterohemorrhagic *E. coli*.

PROGRESS F: The series of studies were undertaken to determine multiple attributes associated with the potential interaction between pH and radiation resistance, including effect of pH (4.0 to 5.5) on radiation resistance, impact of induction of acid tolerance on radiation resistance, potentiation of acid inactivation at refrigeration temperatures by

irradiation, and characterization of injury effects resulting from irradiation and acid exposure. Eight strains (see Objective E) were used to assess the impact of biological diversity. The data are currently being analyzed but initial evaluations demonstrate that induction of acid tolerance significantly increases radiation resistance.

IMPACT/TECH TRANSFER F: In addition to providing data needed to determine the efficacy of irradiation processing for eliminating enteric pathogens from fermented meat products, this work demonstrates that prior pH history can influence the irradiation resistance of foodborne pathogens. Thus, pH history may have to be accounted for when designing irradiation pasteurization processes or characterizing the irradiation resistance of bacteria.

OBJECTIVE G: Effect of acidulant identity on the acid tolerance of enterohemorrhagic *E. coli*.

PROGRESS G: The eight strains used in Objective E were evaluated for acid tolerance using 0.5% citric, lactic, and acetic acids. The cells were grown with or without glucose and then transferred to BHI adjusted to pH 3.0 (0.5% acid) and held for up to 7 hrs at 37 °C. The data are currently being analyzed but initial assessments indicate that the sensitivity of stationary phase cells to acid inactivation is dependent on acidulant identity, prior exposure to an acid environment, and strain identity. Inducing acid tolerance consistently increased the survival of the organism. The relative effectiveness of the acids for most strains was lactic acid > acetic acid > citric acid > hydrochloric acid.

IMPACT/TECH TRANSFER G: These results provide researchers with information needed to more accurately explain how microorganisms survive acidic environments, and they demonstrate that previous hypotheses concerning the mode of action underlying acid tolerance are incomplete until they adequately account for the differences in physiological responses to the different acidulants.

OBJECTIVE H: Develop a new primary model for use for fitting growth curves to microbiological data.

PROGRESS H: A simplified "three-phase linear model" was developed as an alternative means for fitting growth kinetics data. The model was evaluated by comparing its effectiveness with a set of growth data for enterohemorrhagic *E. coli* versus growth curves generated using the Gompertz and Baranyi models. The model was found to be easier to use than the others and provided fits that rivaled the Baranyi model and were clearly superior to the Gompertz model.

IMPACT/TECH TRANSFER H: The method provides microbiologists with a simplified means of fitting growth curves via curve fitting software, and is likely to further accelerate the adoption of this more objective means of fitting growth kinetic data. Even

before the formal publication of the method, it is being used by an increasing number of investigators.

OBJECTIVE I: Develop a means for estimating the dose-response curve.

PROGRESS I: Using data on the incidence of listeriosis and the levels of *Listeria monocytogenes* in smoked salmon in conjunction with the exponential dose-response model, a means for estimating a "worse-case" dose-response relationship for *Listeria monocytogenes* was developed. This approach has the potential for generating dose-response estimates for microorganisms for which human volunteer feeding studies are not likely to be performed.

IMPACT/TECH TRANSFER I: This method has generated a great deal of excitement among scientists and policy-makers involved in establishing microbiological criteria. It is considered a pivotal paper for discussions that will take place at the upcoming Codex Alimentarius Food Hygiene Committee meeting on the establishment of quantitative criteria for *Listeria monocytogenes* in foods.

OBJECTIVE J: Control the protozoan parasite *Toxoplasma gondii* and *Cyclospora cayetanensis* in cooperation with scientists at the Beltsville Agricultural Research Center.

PROGRESS J: *Toxoplasma* oocysts are highly resistant to environmental influences and can survive outside the body for months. We have determined that oocysts can be killed by low doses (0.25 kGy) of gamma-radiation and that vaccination with irradiated oocysts can induce protection in mice. We are conducting studies to determine the applicability of gamma-irradiation for the inactivation of *Cyclospora cayetanensis* on fruit (raspberries and strawberries).

IMPACT/TECH TRANSFER J: These results will be useful to understand the mechanisms of protection against *T. gondii*. These results indicate the potential for using gamma-radiation to inactivate other coccidians such as *Cyclospora cayetanensis* on fruit.

PUBLICATIONS:

Buchanan, R. L. 1996. Egg and egg products. *In* Microorganisms in Foods 6: Microbiology of Food Commodities, Terry Roberts (ed), International Commission on Microbiological Specifications for Foods, Blackie Academic and Professional, London, England. (In press).

Buchanan, R. L. 1996. Fish and fish products. *In* Microorganisms in Foods 6: Microbiology of Food Commodities, Terry Roberts (ed), International Commission on Microbiological Specifications for Foods, Blackie Academic and Professional, London, England. (In press).

Buchanan, R. L. and S. G. Edelson. 1996. Effect of culturing enterohemorrhagic *Escherichia coli* in the presence and absence of glucose on the acid tolerance of stationary phase cells. *Appl. Environ. Microbiol.* (In press).

Dubey, J. P., M. C. Jenkins, and D. W. Thayer. 1996. Irradiation killing of *Toxoplasma gondii* oocysts. *J. Eukaryotic Microbiol.* (In press).

Dubey, J. P., M. C. Jenkins, D. W. Thayer, O.C.H. Kwok, and S. K. Shen. 1996. Killing of *Toxoplasma gondii* oocysts by irradiation and protective immunity induced by vaccination with irradiated oocysts. *J. Parasitol.* (In press).

Kim, A. Y. and D. W. Thayer. 1996. Mechanism by which gamma irradiation increases the sensitivity of *Salmonella typhimurium* ATCC 14028 to heat. *Appl. Environ. Microbiol.* **62**:1759–1763.

Lakritz, L., J. B. Fox, Jr., J. W. Hampson, R. Richardson, K. M. Kohout, and D. W. Thayer. 1995. Effect of gamma radiation on levels of alpha-tocopherol in red meats and turkey. *Meat Sci.* **41**:261–271.

Thayer, D. W. and G. Boyd. 1996. Inactivation of *Shewanella putrefaciens* by gamma irradiation of red meat and poultry. *J. Food Saf.* **16**:151–160.

Thayer, D. W., E. S. Josephson, A. Brynjolfsson, and G. G. Giddings. 1996. Radiation Pasteurization of Food, pp 1–10, CAST Issue Paper No. 7. Council for Agricultural Science and Technology, Ames, IA.

SURFACE PASTEURIZATION

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CRIS Number: **1935–41000–048**
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OBJECTIVE A: Design an in-line machine capable of reducing the level of microbial contamination on the surface of poultry meat in the slaughter line without introducing significant degradation of the quality of the product.

PROGRESS A: A prototype design for an in-line microbial decontamination machine has been conceived, patented, built, and tested. It is designed to treat fresh whole broiler carcasses with steam so quickly that surface organisms are killed; but no appreciable cooking of the meat occurs. The through-put of the machine is sufficiently rapid so that a single machine can serve 4000 birds/hr, which is the rate of a modern slaughter line.

The prototype differs from the feasibility device in that valving of the chickens and valving of the gases are accomplished separately in the prototype. This change reduced deadtime in the cycle by greatly reducing the inertia of the moving part during gas valving.

Four log reduction of applied *Listeria inocuans* was achieved on fresh broilers without cooking by application of 145 °C steam for 25 milliseconds. This was accomplished after removal of air by steam flushing and by evacuation. After treatment, instant surface cooling was accomplished by vacuum.

The prototype will be demonstrated on whole broilers to potential users. This demonstration will use actual pathogen treated broilers. We suggest that the first user be someone who already has a good separation between the chill tank and the cut-up line in their processing plant.

For other uses, the prototype will be exploited to effect surface kills on fresh beef and pork. Surface heating of intact fruits will be tested for its effects on insect eggs and molds. Surface heating of grains will be tried for disinfestation, decortication, and for germ separation.

IMPACT/TECH TRANSFER A: A surface pasteurizer receiving birds from the chill tank and delivering them to a clean cut-up line would comprise an effective barrier between the pathogen-rich slaughter area and the pathogen-free cut-up line. In this way, the present flow of live organisms from the living bird's GI tract into the distribution channels can finally be arrested.

PUBLICATIONS:

Morgan, A. I. 1995. Method of treating raw meat. Canadian Patent 2,116,838. (issued December 10, 1995).

Morgan, A. I. and N. Goldberg. 1996. Design of in-line surface pasteurizer. U.S. Patent Application.

Morgan, A. I., N. Goldberg, E. R. Radewonuk, and O. J. Scullen. 1995. Surface pasteurization of raw poultry meat by steam, pp 215–222. Proceedings of the XII European Symposium on the Quality of Poultry Meat, Zaragoza, Spain.

Morgan, A. I., N. Goldberg, E. R. Radewonuk, and O. J. Scullen. 1996. Surface pasteurization of raw poultry meat by steam. *Lebensm. Wiss. Technol.* (In press).

Morgan, A. I., E. R. Radewonuk, and O. J. Scullen. 1996. Ultra high temperature, ultra short time heating of meat. *J. Food Sci.* (In press).

DETECTION OF PATHOGENIC BACTERIA BY BIOSENSORS

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CRIS Completion Date: **9–01–96 (9–30–98)**

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OBJECTIVE A: Determine the most sensitive internal physiological index for fluorescense detection of a specific bacteria. Develop methods including electrochemical biosensors for detecting bacteria, and improve chemiluminescence processes for detecting bacteria and toxins.

PROGRESS A: Fluorescent Labeling of Bacteria. We used DAPI, a nucleic acid-specific probe, to treat live *E. coli* O157:H7. The labeled cells were then concentrated by magnetic beads coated with the antibody against pathogenic *E. coli*. Captured bacteria were then viewed by a fluorescent light microscope which was interfaced with a computer for imaging analysis. We found that the labeling of live bacteria could be enhanced significantly under certain bioenergetic conditions. This metabolic enhancement was not observed with dead or broken bacteria. Microscopic observations indicated the beads could capture many bacteria. Consequently, the beads also aggregated together when the antibody-specific bacteria were present. The next step is to develop a magnetic slide to align the bacteria-coated beads for an automated imaging capture and analysis.

Immunoelectrochemical Detection of Bacteria. We demonstrated in the past that heat-killed *Salmonella* could be captured by magnetic beads coated with anti-*Salmonella* serum. The captured bacteria were then labeled with an antibody labeled with alkaline phosphatase. After being rapidly concentrated to the surface of a carbon strip electrode with a magnet and incubated with proper phenolic phosphate compounds, the bacteria could then be quantified electrochemically from the phenols produced. This approach was successfully extended to the detection of heat- or irradiation-killed *E. coli* O157:H7 in carcass wash. A new study on capture and detection of bacteria on filter membranes also was advanced. The bacteria, irradiation-killed *E. coli* O157:H7, were first treated with an anti-serum linked with alkaline phosphatase. The treated bacteria were then trapped on filter membranes with 0.2 μm pores. An inverted membrane was placed on the tip of a carbon electrode and then incubated with proper phenolic phosphates. The enzyme (alkaline phosphatase) activity associated with the bacteria was then determined. The future experiments will primarily concentrate on a means to minimize non-specific binding of alkaline-phosphatase conjugated anti-bacteria serum to the membrane and then test it on live bacteria.

Procedure Development of Using Electrochemiluminescence for Bacteria Detection. In March 1996, we obtained a fully automated commercial instrument from IGEN. This instrument utilized magnetic beads to capture bacteria in a similar approach described above, except using automation. The captured bacteria were magnetically held on top of an electrode prior to treating again with another anti-serum antibody linked with the metal Ru (II) complex. The Ru (II) was first oxidized electrochemically to the $^{+}3$ state and then re-reduced back to $^{+}2$ by a reductant in the flow-through solution. The re-reduction of Ru (III) produced chemiluminescence light which was detected by a photo multiplier right above the electrode. With the magnet removed, the bacteria could be washed away by a wash solution to prompt the system for the next sample. The instrument could analyze up to 50 samples in about 2 hrs. We found that this instrument and approach could be used to detect the presence of live *E. coli* O157:H7 spiked in carcass wash solution. A similar MOU with the Army laboratory is being developed.

IMPACT/TECH TRANSFER A: The research, developing means to detect low level pathogens, is directed to an important public health and food safety issue. Successful results may be transformed to methods or devices for detecting low levels of pathogenic microorganisms, such as *E. coli* O157:H7 or *Salmonella*, in meat or poultry. Use of rapid detection in conjunction with existing inspection programs at meat and poultry production facilities would significantly reduce the incidence of microbial contamination and resulting foodborne diseases. To speed up the progress, we signed a MOU with the U.S. Air Force laboratory responsible for developing remote biosensing techniques, at Aberdeen Proving Ground, MD, to exchange research expertise and information. A similar agreement with the U.S. Army research facility at the same site also is being developed.

PUBLICATIONS:

Gehring, A. G., C. G. Crawford, R. S. Mazenko, L. J. Van Houten, and J. D. Brewster. 1996. Enzyme-linked immunomagnetic electrochemical detection of *Salmonella typhimurium*. *J. Immunol. Methods.* (In press).

MILK PASTEURIZATION CONDITIONS FOR *M. PARATUBERCULOSIS*

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CRIS Number: **3625–32000–010**
CRIS Completion Date: **9–30–00**

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OBJECTIVE A: Determine optimal pasteurization conditions for killing *Mycobacterium paratuberculosis* in milk.

PROGRESS A: Evaluation of optimal time/temperature combinations for effective heat inactivation of *M. paratuberculosis* was determined using either a holder-test tube method or a lab-scale industrial pasteurization method. Raw milk obtained from *M. paratuberculosis*-free cows was dispensed into 13 × 100 mm snap-cap polystyrene tubes and placed in a shaking water bath at either 65, 72, 74, or 76 °C, with one tube serving as a temperature control. Milk was inoculated with 10^8 CFU *M. paratuberculosis*/mL (Strains 19698, Ben and Kay) and aliquots removed at each time point for serial dilution and culture on HEYM. Results indicate that the most effective reduction in viable bacterial numbers was achieved at 72 °C, but mean time for optimal killing superseded industry recommendations of 15 sec at that temperature. Studies with the lab-scale pasteurizer were conducted in a similar manner. Raw milk (1–2 liters) inoculated with *M. paratuberculosis* (10^4, 10^6, and 10^8 CFU/mL; strains 19698, Ben, and Kay) was poured into the holding vessel and circulated for 15 sec, 72 °C. Samples were obtained from the output tube at the beginning, middle, and end of the pasteurization run for culture. Results from all experiments conducted with the lab-scale pasteurizer demonstrate that treatment of raw milk inoculated with live *M. paratuberculosis* at 72 °C, 15 sec, effectively killed all the bacteria. Comparison of these two models for testing heat inactivation of *M. paratuberculosis* indicate that the lab-scale pasteurizer more closely simulates industry conditions and that results from those experiments should be given more careful consideration.

IMPACT/TECH TRANSFER A: Milk Producers Federation, Inc. requested the investigation of pasteurization conditions for *M. paratuberculosis*, a potential human pathogen. This information suggests that *M. paratuberculosis* is completely inactivated by industrial pasteurization conditions and is useful for the dairy industry, regulatory veterinarians and consumers.

PUBLICATIONS:

Stabel, J. R., E. Steadham, C. A. Bolin. 1996. Heat inactivation of *Mycobacterium paratuberculosis* in raw milk using holder-test tube method and lab-scale industrial pasteurization method. Proceedings of the 5th International Colloquium on Paratuberculosis, Madison, WI. (In press).

Part IV. RESIDUE DETECTION AND NUTRIENT ANALYSIS

ADVANCED TECHNOLOGIES FOR RESIDUE DETECTION (SFE)

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FSIS Number: **I-2**
CRIS Completion Date: **4–01–98**

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OBJECTIVE A: Develop supercritical fluid extraction (SFE) instrumentation and components suitable for use in regulatory laboratories.

PROGRESS A: The Applied Separations Inc., Allentown, PA, SFE units installed at the FSIS laboratories in Athens, GA and St. Louis, MO, were purchased prior to the introduction of the pump/chiller assembly accessory. This device eliminates the need for the more expensive helium pressurized carbon dioxide (CO_2) cylinders. The presence of helium in these cylinders has been reported by investigators to influence the solubility of some analytes in supercritical carbon dioxide (SC-CO_2), and leads to inconsistencies in analyte recovery from certain matrices.

IMPACT/TECH TRANSFER A: The FSIS SFE pump modules were shipped to ERRC for modification. Pump chiller assemblies, fabricated at ERRC, were installed in these units and bench tested. The modified system was returned to the FSIS laboratories in Athens, GA and St. Louis, MO. All of the FSIS and ARS SFE units are now identical in design facilitating direct transfer of SFE methods and simplifying method verification among the three laboratories.

OBJECTIVE B: Develop a SFE method for the isolation of sulfonamides (SAs) from chicken liver at the regulatory tolerance level.

PROGRESS B: Earlier studies in this laboratory on the isolation of SAs from chicken tissue by SFE were conducted at fortification levels of 1.0 ppm; whereas, the tolerance level is 0.1 ppm. An SFE method, which requires only 1.3 mL of organic solvent/sample was developed for the recovery of three SAs from chicken liver at 0.1 ppm. Samples were extracted at 680 bar (10,000 psi) and 40 °C using non-modified CO_2 and were collected free of coextracted artifactual material on an in-line neutral alumina sorbent bed. Then,

eluates from the sorbent beds were directly analyzed by one-step post SFE sequence. Recoveries of sulfamethazine (SMZ), sulfadimethoxine (SDX) and sulfaquinoxaline (SQX) spiked tissue were 86%, 92%, and 79%, respectively. Comparable results were obtained for liver samples fortified at the 0.05 ppm level using similar SFE experimental conditions.

IMPACT/TECH TRANSFER B: Samples of chicken tissues containing normally incurred SA were received for a comparative study of SFE and a conventional solvent-based method. At the completion of these trials the SFE method will be transferred to FSIS for further evaluation.

OBJECTIVE C: Apply SFE for the isolation of drug residues in eggs.

PROGRESS C: SFE was applied to analysis of three SAs in eggs under conditions similar to that employed for SAs in meat tissue by Dr. Parks. Whole egg (1 g) was mixed with Hydromatrix and added to an extraction vessel containing neutral alumina. The sample was extracted at 40 °C with SC-CO_2, without the use of a solvent modifier, at 10,000 psi (680 bar) and a flow rate of expanded gas of 3.0 L/min into a total volume of 120 L. In-line trapping of the SAs on the alumina sorbent and their elution with the HPLC mobile phase solvent system (phosphate buffer-MeOH) and HPLC determination (C_{18} column, UV detection at 265 nm) gave excellent results. Recoveries from fortified eggs ($n = 6$) at the 0.1 ppm level were: SMZ, 99.5 ± 2.2%; SDM, 87.8 ± 6.0% and SQX, 97.6 ± 2.5%. The limit of detection was 0.025 ppm. In-line SA trapping resulted in higher recoveries and gave cleaner chromatograms than those obtained by off-line trapping. These SAs are more readily extracted from eggs than noted in previous studies on meat tissue, suggesting a different matrix-SA interaction taking place.

IMPACT/TECH TRANSFER C: The same FSIS laboratory carrying out SFE for SAs on meat tissue will now be able to perform these analyses on eggs. The method will be transferred to FSIS for evaluation after studies are completed on eggs with incurred sulfamethazine. Egg laying chickens were fed encapsulated SMZ for this purpose by FDA, CVM personnel.

OBJECTIVE D: Apply SFE for the isolation of pesticide residues in eggs.

PROGRESS D: Supercritical carbon dioxide extraction of 16 common organochlorine pesticides (OCPs) was carried out on liquid, whole chicken eggs. The OCPs included: aldrin, α-, β-, δ- and γ-BHCs, 4,4'-DDD, 4,4'-DDE and 4,4'-DDT, dieldrin, endosulfan I, II and sulfate, endrin, endrin aldehyde, heptachlor and heptachlor epoxide. The extraction vessel was packed with a mixture of 1.0 g whole egg mixed with 4.0 g Hydromatrix and 1.5 g Hydromatrix alone. The SFE conditions were: 680 bar (10,000 psi), 40 °C, SC-CO_2 flow rate 3.0 L/min into a total volume of 120 L. Organochlorine pesticides were trapped off-line on an SPE cartridge containing Florisil, eluted with a pentane-acetone mixture

before analysis by capillary GC-electron capture detector (EC). The chromatograms were free of background interfering peaks. Recoveries were obtained from three levels of fortification, 0.05, 0.1 and 0.2 ppm; no significant difference ($p > 0.05$) in results were found. From five samples, fortified at the 0.1 ppm level, OCP recoveries ranged from 76.0 to 102.9%, with an average of 88.2%. Off-line trapping was found to be superior to in-line trapping insofar as recovery, reproducibility and cleanliness of extract. Most noteworthy, was the effective extraction of several polar OCPs by SC-CO_2 without the use of solvent modifiers.

IMPACT/TECH TRANSFER D: This SC-CO_2 method permits the rapid isolation of OCPs free of co-extracted lipids, without the time-consuming, solvent intensive extraction and concentration steps required by the currently employed techniques. This method effectively extracts many of the most carcinogenic and environmentally persistent OCP contaminants. The method will be transferred to an FSIS laboratory for evaluation and use.

OBJECTIVE E: Investigate the use of automated on-line microdialysis for analyte sample preparation (analyte extraction and concentration) prior to HPLC determination.

PROGRESS E: The isolation of fluoroquinolones, flumequine and oxolinic acid, from fortified chicken liver was achieved using liquid–liquid extraction, aqueous on-line microdialysis, and trace enrichment. Dialysis, trace enrichment and column switching were performed using a Gilson ASTED system. Analysis of the isolated compounds in the tissue extracts was made using reversed-phase HPLC and fluorescence detection. This procedure yielded excellent recoveries at 50 ng/g (92%, 9% RSD) and 10 ng/g (94%, 10% RSD) spiking levels for flumequine and at the 25 ng/g (86%, 5% RSD) and 5 ng/g (99%, 6% RSD) spiking levels for oxolinic acid. Clean chromatograms were obtained, allowing easy detection of 10 ng/g flumequine and 5 ng/g oxolinic acid. The virtues of this method are its low organic solvent consumption, short sample processing time, small sample volume, automation and high sample throughput, thus making this method suitable for regulatory use.

IMPACT/TECH TRANSFER E: The excellent results achieved for the recovery of flumequine from chicken liver by on-line microdialysis demonstrates the potential of this technique for routine monitoring. Studies with samples containing incurred fluoroquinolone in chicken tissue are planned to further develop this technique prior to transfer to FSIS.

The on-line microdialysis project was begun in June, 1995 by a Research Associate, then a member of the research group. Work on this project continued to October, 1995 when the project was terminated due to the resignation of the Research Associate. Because of the potential impact of this technology, a specific cooperative agreement (SCA) was made with Drexel University to obtain a graduate student to work on this program until a

permanent SY position is approved. The student will begin work on this research project in September, 1996.

IMPACT/TECH TRANSFER E: Plans have been made to obtain poultry tissue samples containing incurred sarafloxacin from collaborating FDA, CVM personnel. These samples will be analyzed by both on-line microdialysis and a solvent based method. The data from this evaluation will be transferred to FSIS for their consideration.

OBJECTIVE F: Evaluate supercritical fluid chromatography (SFC) for the separation and detection of trace levels of pesticide and drug residues.

PROGRESS F: A specific cooperative agreement was initiated with Virginia Polytech. for the above purpose. Initial experiments have been carried out using a commercially available chemiluminescence nitrogen detector (CLND) in combination with a packed HPLC column. Instrumental parameters such as, decompressed CO_2 flow rate, modifier content and detector ozone flow rate were modified to optimize detector sensitivity. For two single nitrogen-containing compounds, detectable levels of 213 pg and 448 pg of nitrogen (N) were obtained. This work was expanded to include sulfonamides containing up to 4 nitrogen atoms. At present, 5 ng of sulfamethazine can be detected. This detection level is due to a current 5:1 flow splitter. Virginia Tech personnel are working with Antek Instruments, the manufacturer of this CLND to improve sensitivity by modifications in the detector design.

IMPACT/TECH TRANSFER F: The application of this technology, as with the others, is solvent-sparing and reduces the generation of hazardous waste. In addition, residues and/or their metabolites, heretofore difficult to analyze by GC because of their thermal instability will now be capable of analysis. Since this agreement has only recently been initiated, no technical transfer has taken place.

PUBLICATIONS:

Eng, G. Y., R. J. Maxwell, E. G. Piotrowski, and W. Fiddler. 1996. Analysis of flumequine and oxolinic acid in fortified chicken tissue using on-line microdialysis and fluorescence HPLC. *J. Chromatogr.* (In press).

Eng, G. Y., M. B. Medina, and L. E. Jones. 1995. Characterization of an immobilized protein matrix for use in the isolation and detection of beta-lactam antibiotics. *Biotechnol. Appl. Biochem.* **22**:129–144.

Maxwell, R. J. and A. R. Lightfield. 1996. Multiresidue method for the supercritical carbon dioxide recovery of sulfonamides from chicken liver at low ppb levels. *J. Chromatogr.* (In press).

Maxwell, R. J., A. R. Lightfield, and O. W. Parks. 1996. Approaches to the supercritical fluid extraction of sulfonamides from beef and pork livers, p C-14, Vol 7. Proceedings of the 7th International Symposium on Supercritical Fluid Chromatography and Extraction, Indianapolis, IN.

Maxwell, R. J. and J. F. Morrison. 1996. Supercritical fluid extraction as a sample preparation tool in analytical toxicology. *In* Handbook of Analytical Therapeutic Drug Monitoring and Toxicolgy, S.H.Y. Wong and I. Sunshine (eds), CRC Press, Boca Raton, FL. (In press).

Maxwell, R. J., O. W. Parks, A. R. Lightfield, and A.A.M. Stolker. 1996. Recovery of trace levels of veternary pharmaceuticals from biological matrices, p L-16, Vol 7. Proceedings of the 7th International Symposium on Supercritical Fluid Chromatography and Extraction, Indianapolis, IN.

Parks, O. W., A. R. Lightfield, and R. J. Maxwell. 1995. Effect of sample matrix dehydration during SFE on recoveries of drug residues from fortified chicken liver. *J. Chromatogr. Sci.* **33**:654–657.

Parks. O. W., R. J. Shadwell, A. R. Lightfield, and R. J. Maxwell. 1996. Determination of melengestrol acetate in supercritical fluid–solid phase extracts of bovine fat tissue by HPLC-UV and GC-MS. *J. Chromatogr. Sci.* **34**:353–357.

DEVELOP IMMUNOCHEMICAL-BASED RESIDUE METHODS FOR ANALYSIS OF VETERINARY DRUG AND PESTICIDE RESIDUES IN FOOD ANIMALS

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CRIS Number: **6202–42000–009**
FSIS Number: **I-2**
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OBJECTIVE A: Develop, evaluate, and provide confirmatory testing of monoclonal antibody-based immunoassays useful for either on-the-farm or in-the-plant (processing), and for laboratory based analysis of pesticide/drug residues in animal products and body fluids.

PROGRESS A: Monoclonal antibody based immunoassays are in development for a number of compounds including: halofuginone, hygromycin B, pirlimycin, fumonisin, furosemide, ceftiofur, salinomycin, monensin, and various sulfa drugs (e.g., sulfamethazine). Most of these have been identified by FSIS as being important for development of rapid immunoassays. The immunoassays for halofuginone, ceftiofur, salinomycin, and hygromycin B are the most complete.

Halofuginone (Hal): The halofuginone immunoassay is formatted as a competition enzyme-linked immunosorbent assay (cELISA) and is capable of detecting halofuginone in the 1 to 10 ppb range. A simplified sample preparation method for analysis of chicken livers has been developed that greatly reduced the sample preparation time compared to the currently used FSIS HPLC method. The new method utilizes an aqueous buffer for extraction and the above cELISA for detection and quantification. Analyses of spiked chicken liver samples containing 0.04 to 0.32 ppm halofuginone have been completed and sample recoveries of 85.7 to 106.9% were observed. A detailed protocol for this immunoassay method describing all reagents and each step of the method has been prepared and submitted to scientists at the FSIS Midwestern Laboratory (St. Louis, MO). In collaboration with FSIS scientists at the Midwestern Laboratory, analyses of identical samples using both the ARS immunoassay and the published FSIS chromatographic method have been completed. This sample set included both fortified chicken livers and samples containing incurred residues prepared at our laboratory. Samples spiked at 100, 160 and 180 ppb halofuginone (the range measured by the FSIS chromatographic method) resulted in recoveries of 81.1, 78.8 and 82.2% respectively by the HPLC method, and 90.0, 84.3 and 62.4% by the ARS cELISA method. The incurred residues were generated in chickens, and samples were taken at various times following dosing with halofuginone. The results for the incurred residue study are summarized in Table 1.

Table 1. Incurred halofuginone levels (ppb) in chicken liver.

Control	2 hr	6 hr	24 hr	96 hr
The FSIS HPLC method:				
ND*	698.5 ± 100.3	1031.2 ± 114	333 ± 80.1	< 50 (16 ± 4)
The ARS cELISA method:				
ND	932.6 ± 196.3	1018.2 ± 36.6	200.5 ± 24.6	< 75 (30 ± 12)

* ND = not detected

IMPACT/TECH TRANSFER Hal: This immunoassay is being transferred to FSIS scientists, St. Louis. In addition, these antibodies also have been supplied to a private kit manufacturer who is evaluating them for their application to a commercial immunoassay following a request from the drug manufacturer for a rapid test. A number of manuscripts describing this assay have been published.

Ceftiofur (Cef): Ceftiofur is an FDA approved veterinary cephalosporin antibiotic for the treatment of respiratory diseases in cattle, horses, and swine. We have developed a monoclonal antibody utilizing as immunogen a protein conjugate of the ceftiofur metabolite, desfurylceftiofur. The monoclonal antibody forms the basis of a competition ELISA. Experiments with raw milk obtained from the bulk tank indicate that ceftiofur can be detected in milk samples using the cELISA without the need for any extraction or cleanup steps. The cELISA performance also was evaluated using spiked milk samples from individual cows. In these tests the average recovery was 100.5% ranging between 80 to 121%. Incurred residues in milk samples also were evaluated using the cELISA and the observed values were similar to those obtained in studies by others using radiolabeled drug. These same incurred residues were sent to other ARS laboratories for use in development of an HPLC method for ceftiofur in milk. Our initial findings suggest that the cELISA detects not only ceftiofur but the major metabolite of ceftiofur, desfurylceftiofur and conjugates of desfurylceftiofur. Thus, unlike many HPLC methods which only measure parent or a specific metabolite, the cELISA results should be expressed as ceftiofur equivalents since it appears to detect metabolites and protein conjugates. In addition to the above work with milk, we have developed a simplified extraction method for ceftiofur analysis in beef kidney. We have attempted to incorporate as much of the standard FSIS protocol as possible into our method in order to facilitate transfer of the assay to the appropriate FSIS group. Recoveries near 100% were observed in spike-recovery studies from beef kidney material.

IMPACT/TECH TRANSFER Cef: We have prepared a detailed protocol for our method and packaged all of the necessary immunochemical reagents into a convenient "kit" form which we have made available to the FSIS Scientist (Antibiotic Residues Section, Biological Contaminants Branch, BARC-East). We continue to work with FSIS in order to transfer this assay to their laboratories. This antibody was developed under an existing CRADA and it is currently being formatted into a commercial immunoassay kit by

our CRADA partner. A patent for the monoclonal antibody is pending and our CRADA partner is in the final stages of negotiating a royalty bearing license with ARS for use of these antibodies.

Salinomycin (Sal): The salinomycin immunoassay we developed also is formatted as a competition ELISA, with sensitivities in the low ppb range. This is a simple, rapid assay that is able to detect salinomycin in chicken liver. Sample preparation is simple and does not require the use of organic solvents. Liver samples are simply homogenized in buffer, diluted, and assayed. Studies using fortified liver samples resulted in recoveries of 80–100%. Incurred liver samples have been tested using both our ELISA method and an HPLC method also developed in our laboratory. Good correlation's between the two methods were obtained. However, the ELISA was far more sensitive than the HPLC. These studies recently have been published.

IMPACT/TECH TRANSFER Sal: A U.S. patent covering the salinomycin ELISA has been issued (Patent Number 5,466,784). This antibody has been exclusively licensed to Neogen Corp., Lansing, MI, and they are currently marketing a rapid diagnostic immunoassay kit for detection of salinomycin in feeds.

Sulfa Drugs (SD): A series of monoclonal antibodies have been developed that bind various sulfa drugs, specifically, sulfamethazine, sulfadimethoxine, and sulfathiazole. These sulfa compounds were indicated as high priority by FSIS. To date we have completed our studies with the anti-sulfathiazole monoclonal antibodies and formatted these into a rapid cELISA. The cELISA has been applied to the analysis of chicken liver samples. Both spiked liver samples and samples containing incurred residues produced at our facility have been evaluated with the above cELISA and with an HPLC method we adapted from published reports. Our aim is to complete evaluation of the anti-sulfadimethoxine and anti-sulfamethazine antibodies and incorporate these into a single assay, a cELISA or other appropriate immunoassay format, capable of measuring each of these three commonly used sulfa drugs.

IMPACT/TECH TRANSFER SD: Development of the above immunoassays was initiated in collaboration with a CRADA partner. These antibodies are being formatted by the CRADA partner to reflect their existing immunoassay product line.

Other Compounds (OC): We have developed monoclonal antibodies and an ELISA for fumonisin. The assay detects fumonisin B_1, B_2 and B_3. Antibody cross reactivity has been correlated with computer generated energy-minimized molecular models of these mycotoxins. Molecular modeling of the stereostructure of fumonisin B_1 was completed with the backbone stereochemical assignments according to Hoye *et al.* (1994) and the stereochemical assignments of the acid side chains determined as (*S*) by Shier *et al.* (1995). Further molecular modeling work on the stereoisomer of FB_1 with the stereochemical assignments of the acid side chains in the (*R*) configuration and the stereochemical assignments of FB_2 will be completed in the near future. These same antibodies have been made available to a number of companies for evaluation. This past

year we have developed a group of monoclonal antibodies that bind various fluoroquinolones including sarafloxacin, enrofloxacin and norfloxacin. Samples containing incurred residues of these compounds have been prepared and these monoclonal antibodies are being formulated into a rapid immunoassay for detection of these newly approved veterinary drugs. We continue our collaboration with Dr. Bruce Hammock, University of California-Davis in an effort to produce anti-dioxin monoclonal antibodies and analogs for dioxin. We have studied the response of an existing set of anti-dioxin monoclonal antibodies to a group of new dioxin haptens synthesized in Dr. Hammocks' laboratory. These studies also have utilized low-energy molecular models of the polychlorinated dioxins and furans. We anticipate generating a new set of anti-dioxin monoclonal antibodies. Finally, we have generated and reported previously on a group of monoclonal antibodies to hygromycin B.

IMPACT/TECH TRANSFER OC: A patent has been allowed by the U.S. Patent Office covering the anti-hygromycin monoclonal antibodies. These anti-hygromycin B monoclonal antibodies are the subject of ongoing negotiations between ARS and a private immunoassay kit manufacturer for a royalty bearing license for the use of these reagents.

OBJECTIVE B: Development of classical analytical methodologies (e.g., HPLC, GLC) for the analysis of pesticide/drug residues to provide confirmatory testing of the immunoassays.

PROGRESS B: Traditional analytical methods continue to be developed in our laboratory to support our studies on development of immunoassays. Previously published studies include an extraction and HPLC method for detecting halofuginone in chicken serum and an HPLC method for detection of salinomycin residues in chicken liver tissue. An HPLC method also was developed for detecting furosemide in raw milk. Recently, we have developed HPLC methods for analysis of sulfonamide residues in chicken liver in support of the above described immunoassay research. Results from these latter studies have been submitted for publication.

IMPACT/TECH TRANSFER B: These studies have impacted our ability to validate the immunoassays developed in objective A.

OBJECTIVE C: Develop molecularly imprinted polymers for extraction, cleanup, and detection of residues in foods.

PROGRESS C: The development of molecularly imprinted polymers as tools in analytical chemistry is a new and exciting area of study. We have generated a series of molecular imprints including imprints to, atrazine, salinomycin, halofuginone, and ceftiofur. We have studied the application of these imprinted polymers as stand-alone assays, comparing them to antibody methods, and as chromatographic supports. Recent studies demonstrated that molecularly imprinted polymers were highly effective reagents

for rapid, one-step, selective cleanup and concentration of residues from complex biological matrices (chicken liver samples). This approach, designated molecularly imprinted solid phase extraction (MISPE), improved recoveries of atrazine residues from chicken liver samples as measured using either an HPLC or an ELISA method. We anticipate future studies aimed at incorporating MISPE into an automated, multiple column high pressure liquid handling system capable of utilizing on-line immunoassays for final detection.

IMPACT/TECH TRANSFER C: These studies have been published in scientific journals, chemical industry trade magazines, and presented at major scientific meetings. In addition, we have supplied imprinted polymers to collaborators in Russia who are developing fluorescent-based methods for residue detection.

OBJECTIVE D: Produce food-animal tissues and body fluids containing specified levels of incurred residues of veterinary drugs, pesticides, or other chemicals for use by FSIS in the development and validation of analytical methods.

PROGRESS D: Four studies involving incurred drug residues in animal tissues and fluids were completed. 1) Phase II of a sulfamethazine and sulfathiazole study in pigs was complete and liver, kidney and muscle tissues were sent to the USDA-FSIS Eastern Laboratory (Athens, GA). 2) Phase III of a sulfathiazole study in pigs also was completed and tissues were sent to the FSIS Eastern Laboratory. 3) A ceftiofur incurred residue study in cattle was completed and materials sent to the FSIS Microbiology Division, BARC-East. 4) Finally, cows were dosed with cephapirin and tissue samples sent to ARS BARC-West. Studies underway, requested by the FSIS Midwestern Laboratory, St. Louis, MO, include production of poultry tissues and fluids containing incurred residues of danofloxacin and bovine tissues and fluids containing enrofloxacin. In addition to these studies, incurred residues have been generated for use in objective A above including milk samples containing residues of furosemide and ceftiofur.

IMPACT/TECH TRANSFER D: Reports detailing each incurred project were prepared and forwarded along with the samples to the FSIS or the FSIS designated agency.

PUBLICATIONS:

Beier, R. C. 1995. Role of Metabolism and the toxic response of some naturally occurring chemicals in food, pp. 309–332. *In* The Mammalian Metabolism of Agrochemicals, D. H. Hutson and G. D. Paulson (eds), Vol. 8: Progress in Pesticide Biochemistry and Toxicology. John Wiley & Sons Ltd., Chichester, England, 340 pp.

Beier, R. C. 1996. Naturally occurring biologically-active non-nutrients in foods. *Manejo Integrado De Plagas (Costa Rica)* **39**(March):30–36.

Beier, R. C., M. H. Elissalde, and L. H. Stanker. 1996. Molecular modeling studies of the fumonisin mycotoxins, pp 368–387. *In* Immunoassays for Residue Analysis: Food Safety, R. C. Beier and L. H. Stanker (eds), ACS Symposium Series 621. American Chemical Society, Washington, DC, 528 pp.

Beier, R. C., L. D. Rowe, M.I.A. Nasr, M. H. Elissalde, B. G. Rose, and L. H. Stanker. 1996. Detection of halofuginone residues in chicken serum by a monoclonal-based immunoassay and high-performance liquid chromatography. *Food Agric. Immunol.* **8**(1):11–17.

Beier, R. C. and L. H. Stanker (eds). 1996. Immunoassays for Residue Analysis: Food Safety, ACS Symposium Series 621. American Chemical Society, Washington, DC, 528 pp. (Book)

Caldecott, K. W., J. D. Tucker, L. H. Stanker, and L. H. Thompson. 1995. Characterization of the XRCC-DNA ligase III complex *in vitro* and its absence from mutant hamster cells. *Nucleic Acids Res.* **23**:4836–4843.

Gray, M. L., C. Young, L. H. Stanker, and D. I. Bounous. 1995. Measurement of serum haptoglobin in neonatal farm-raised and bob veal calves using two immunoassay methods. *Vet. Clin. Pathol.* **25**:38–42.

Ivie, G. W. and R. C. Beier. 1996. Isopimpinellin is not phototoxic in a chick skin assay. *Photochem. Photobiol.* **63**(3):306–307.

Kamps-Holtzapple, C. and L. H. Stanker. 1996. Development of recombinant ScFvs recognizing potato glycoalkaloids, pp 499–510. *In* Immunoassays for Residue Analysis: Food Safety, R. C. Beier and L. H. Stanker (eds), ACS Symposium Series 621. American Chemical Society, Washington, DC, 528 pp

Kurtz, D. A., J. H. Skerritt, and L. H. Stanker (eds) 1995. New Frontiers in Agrochemical Immunoassay, AOAC International, Arlington, VA, 317 pp. (Book)

Muldoon, M. T., M. H. Elissalde, R. C. Beier, and L. H. Stanker. 1996. Comparison of ELISA and HPLC methods for the detection and quantification of salinomycin in chicken liver tissue, pp 109–121. *In* Immunoassays for Residue Analysis: Food Safety, R. C. Beier and L. H. Stanker (eds.), ACS Symposium Series 621. American Chemical Society, Washington, DC, 528 pp.

Muldoon, M. T. and L. H. Stanker. 1996. Plastic antibodies: Molecularly-imprinted polymers. *Chem. Ind.* **16**(March): 204–207.

Nigg, H. N. and R. C. Beier. 1995. Evaluation of food for potential toxicants, pp. 192–201. Proceedings of the 10th Annual Symposium in Plant Physiology. *In* Current Topics in Plant Physiology, D. L. Gustine and H. E. Flores (eds), Vol. 15: Phytochemicals and Health. American Society of Plant Physiologis's Series, Rockville, MD, 348 pp.

Rose, B. G., S. A. Buckley, C. Kamps-Holtzapple, R. C. Beier, and L. H. Stanker. 1996. Ceftiofur sodium: Monoclonal antibody development and cross-reactivity studies with structurally related cephalosporins. *J. Agric. Food Chem.* **44**:622–627.

Rose, B. G., S. A. Buckley, C. Kamps-Holtzapple, R. C. Beier, and L. H. Stanker. 1996. Molecular modeling studies of Ceftiofur: A tool for hapten design and antibody production, pp 82–98. *In* Immunoassays for Residue Analysis: Food Safety, R. C. Beier and L. H. Stanker (eds), ACS Symposium Series 621. American Chemical Society, Washington, DC, 528 pp.

Rose, B. G., C. Kamps-Holtzapple, and L. H. Stanker. 1995. Competitive indirect ELISA for ceftiofur sodium and the effect of different immunizing and coating antigen conjugates. *Bioconjugate Chem.* **6**:529–535.

Shimer, G. D., L. H. Stanker, M. H. Elissalde, C. B. Bird, D. E. Dixon, M. C. Richards, D. J. Ledden, and B. M. Miller. 1996. Immunochemical detection of the anticoccidial salinomycin in poultry feed, pp 99–108. *In* Immunoassays for Residue Analysis: Food Safety, R. C. Beier and L. H. Stanker (eds), ACS Symposium Series 621. American Chemical Society, Washington, DC, 528 pp.

Stanker, L. H. and R. C. Beier. 1996. Introduction to immunoassays for residue analysis: Concepts, formats, and applications, pp 2–16. *In* Immunoassays for Residue Analysis: Food Safety, R. C. Beier and L. H. Stanker (eds), ACS Symposium Series 621. American Chemical Society, Washington, DC, 528 pp.

Stanker, L. H., C. Kamps-Holtzapple, R. C. Beier, C. E. Levin, and M. Friedman. 1996. Detection and quantification of glycoalkaloids: Comparison of enzyme-linked immunosorbent assay and high-performance liquid chromatography methods, pp 243–255. *In* Immunoassays for Residue Analysis: Food Safety, R. C. Beier and L. H. Stanker (eds), ACS Symposium Series 621. American Chemical Society, Washington, DC, 528 pp.

Vanderlaan, M., L. H. Stanker, B. Watkins, P. Petrovic, and S. Gorbach. 1995. Method for Immunodiagnostic Detection of Dioxins at Low Concentrations. U.S. Patent Number 5,429,925.

Wittum, T. E., C. R. Young, L. H. Stanker, D. D. Griffin, L. J. Perino, and E. T. Littledike. 1996. Haptoglobin response to clinical respiratory disease in feedlot cattle. *Am. J. Vet. Res.* **57**:646–649.

Young, C. R., P. D. Eckersall, P. K. Saini, and L. H. Stanker. 1996. Validation of immunoassays for bovine haptoglobin. *Vet. Immunol. Immunopath.* **49**:1–13.

Young, C. R., T. E. Wittum, L. H. Stanker, L. J. Perino, D. D. Griffin, and E. T. Littledike. 1996. Serum haptoglobin levels in a population of feedlot cattle. *Am. J. Vet. Res.* **57**:138–141.

ADVANCED TECHNOLOGIES FOR RESIDUE DETECTION (ELISA)

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OBJECTIVE A: Develop assays for hygromycin B with low ppb detectability.

PROGRESS A: Two assays were developed using the BIAcore biosensor, and fluorescent-latex particle immunoassays. An indirect immuno-competitive biosensor assay was optimized. Hygromycin B was immobilized on the sensor chip and the hygromycin B in samples or standards were allowed to bind off-line with a known and equal amount of anti-hygromycin B antibody. The mixtures were automatically injected into the BIAcore, allowing the binding of the "free" unbound IgG for 5 min. Results in spiked kidney extract show the limit of detection (LOD) at 25 ppb. Deproteination or inactivation of aminoglycoside binding proteins may improve the sensitivity. A fluorescent-latex particle assay also was optimized for detection of hygromycin B from 5–100 ppb. The samples or standards were pre-incubated for 20 min with hygromycin B antibody immobilized on the latex particles; then the fluorescent tracer (hygromycin B labeled with FITC, a fluorescein compound) was added. The latex bound complexes were separated by centrifugation and the "free" hygromycin B-FITC in the supernate was measured in a 96-well fluorometer. Results with spiked kidney extract show detectability at 5–100 ppb. Also, a hygromycin B immunoaffinity column was optimized for the isolation of hygromycin B and the drug was detectable at 50 ppb by direct fluorescent analysis of the hygromycin-fluorescamine derivative. The column can be used at least 10 times.

IMPACT/TECH TRANSFER A: This assay can be used to detect unidentified microbial inhibitors (UMIs.) The reagents, FITC-hygromycin B and antibody-latex particles can be prepared in batch quantities by Diagnostic Specialties (Metuchen, NJ) at minimal cost, e.g. less than $500 for a 6 month supply. The hyromycin B-affinity column can be prepared at a cost of $25 for each column.

OBJECTIVE B: Develop assays for spectinomycin with low ppb detectability.

PROGRESS B: The spectinomycin sheep antisera was produced and its binding and dissociation kinetics were determined with the BIAcore biosensor. The spectinomycin IgG had an apparent dissociation and binding rates of 10^{-6} and 10^{5}, respectively. The apparent binding (affinity) constant, K, was 10^{11}. This antibody was utilized for the

fluorescent-latex particle assay. The samples or standards were pre-incubated for 20 min with the spectinomycin antibody immobilized on the latex particles; then the fluorescent tracer (spectinomycin labeled with DTAF, a fluorescein compound) was added. The latex bound complexes were separated by centrifugation and the "free" spectinomycin-DTAF in the supernate was measured in a 96-well fluorometer. Results with spiked kidney extract show detectability at 5–100 ppb. The BIAcore also can be used for the spectinomycin assay.

IMPACT/TECH TRANSFER B: This assay can be used to detect UMIs. The reagents, DTAF-spectinomycin and antibody-latex can be prepared in batch quantities by Diagnostic Specialties (Metuchen, NJ) at minimal cost, e.g., less than $500 for a 6 month supply.

OBJECTIVE C: Develop method for screening analysis and classification of β-lactam antibiotics in tissue samples.

PROGRESS C: Lactamase II type and Bacto® Penase were utilized to selectively digest the β-lactams. The hydrolyzed β-lactams were detected by a microbial inhibition assay and an enzyme-linked immunosorbent assay (ELISA) for β-lactams, and also by a specific assay for ceftiofur. Three levels (10, 50, 100 ppb) of β-lactams were spiked into kidney extracts and digested with 3 different concentrations of the enzymes and the unhydrolyzed and hydrolyzed drugs were tested with the 3 assays. This approach can separate the cephalosporin family from the penicillin family. Results indicate that, ceftiofur and cephapirin produce unique patterns after enzyme digestion. Hetacillin and cloxacillin also may be distinguishable from other penicillin compounds. Forty tissue samples containing incurred β-lactams, UMIs and market samples were analyzed. Further analyses of 26 UMI samples are in progress.

IMPACT/TECH TRANSFER C: The use of this method to supplement the 7-plate assay can identify, ceftiofur and cephapirin. The tentative classification of penicillin compounds can expedite the identification and quantification of the β-lactams by HPLC.

PUBLICATIONS:

Medina, M. B. 1996. Latex particle concentration fluorescence assays for rapid detection of trace levels of antibiotics, pp 132–143. *In* Veterinary Drug Analysis: Food Safety, W. A. Moats and M. B. Medina (eds), ACS Symposium Series 636, American Chemical Society, Washington, DC, 192 pp.

Moats W. A. and M. B. Medina (eds). 1996. Veterinary Drug Analysis: Food Safety. ACS Symposium Series 636, American Chemical Society, Washington, DC, 192 pp.

DEVELOP MULTIPLE RESIDUE IDENTIFICATION METHODS FOR TESTING FOOD ANIMAL TISSUES

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FSIS Number: **I-2**
CRIS Completion Date: **1–01–97**

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OBJECTIVE A: Develop simple and rapid physical-chemical procedures for detection and confirmation of antibiotic residues in animal products at levels of concern to regulatory agencies, and verify methods using incurred residues from treated animals.

PROGRESS A: Studies were continued on adapting the multi-residue procedure for determination of β-lactam antibiotic residues in milk to residues in tissues using HPLC fractionation for cleanup. Penicillin-G degraded more or less rapidly in beef kidney and liver homogenates resulting in low and erratic recoveries. Better results were achieved by extracting tissue directly with acetonitrile and tetraethylammonium chloride. Several combinations of HPLC columns and mobile-phases were evaluated for analysis of fractions. The best results were obtained with an Inertsil ODS-2 column with KH_2PO_4-H_3PO_4 buffers. Residues could be determined at levels as low as 0.01 ppm in beef and pork muscle, liver and kidney.

Studies were continued on methods for determination of tetracycline antibiotics in milk. The extraction method was modified to give more consistent recoveries. The procedure was simplified by using direct evaporation of acetonitrile extracts. The HC1 concentration in the extracts was optimized so that degradation was minimal during evaporation of solvents. The Inertsil ODS-2 column was compared with the PLRP-S column for determination of tetracycline antibiotics. The PLRP-S column gave better separation from interferences in sample extracts.

IMPACT/TECH TRANSFER A: FSIS was regularly informed of progress. Training in the procedures was given to scientists from North Carolina State University.

PUBLICATIONS:

Moats W. A. and M. B. Medina (eds). 1996. Veterinary Drug Analysis: Food Safety. ACS Symposium Series 636, American Chemical Society, Washington, DC, 192 pp.

METHODS DEVELOPMENT FOR ANALYSIS OF RESIDUES IN MEAT AND OTHER AGRICULTURAL COMMODITIES

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CRIS Number: **1270–42000–003**
CRIS Completion Date: **12–01–97**

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OBJECTIVE A: Develop quantitative multiresidue methods of analysis for chemical residues in meat and other agricultural products suitable for regulatory purposes, emphasizing the use of chromatography, fluorescence, ion-trap mass spectrometry, supercritical fluid extraction, accelerated solvent extraction, and capillary electrophoresis to maximize recoveries of analytes while minimizing or eliminating matrix interferences.

PROGRESS A: Supercritical Fluid Extraction (SFE). Research has continued to further streamline, optimize, and test our SFE method for more pesticides and commodities. A thorough study of trapping, elution, and clean-up for multiple pesticides was performed. SFE recoveries, trap elution volumes, reproducibilities, limits of detection, and selectivity were compared for 58 diverse pesticides in 3 commodities (apple, orange, and sweet potato) using 4 solid-phase sorbents (ODS, diol, Porapak-Q, and Tenax), and 4 elution solvents (acetone, acetonitrile, ethyl acetate, and methanol). Results showed that trapping with ODS and elution with acetone gave the most reproducibly high recoveries. More selectivity could be gained for selected pesticides through the use of other trap/solvent combinations, but GC/ion trap MS detection proved able to quantify and confirm low level pesticide residues in the extracts in the SFE extracts.

Accelerated Solvent Extraction (ASE). In February 1996, a Dionex ASE 200 production model replaced the prototype instrument used earlier in our lab. ASE was used in studies to extract pesticides from soybean, produce, and meat samples. Experiments tested different solvents, including water, at different pH, temperature, pressure, and extraction volumes. Water was a poor extraction solvent even at 200 °C for the majority of pesticides tested. Organic solvents at mild conditions were able to extract the pesticides from the matrices, but water removal, evaporation, and clean-up steps were needed in most cases before GC or CE analysis.

Comparison of SFE and ASE was performed using Hydromatrix (HMX) and a fibrous cellulose powder, CF-1, as drying agents. CF-1 retained more water in SFE than HMX, but the Whatman powder was more expensive and harder to work with than HMX. A study was conducted to demonstrate the effect of sample water content in SFE and ASE

versus pesticide solubility in water. Results for pesticides with solubilities in water of ≥ 1 mg/L were similar in SFE and ASE. For less soluble pesticides, SFE recoveries began to decrease when water was present in the sample. ASE achieved high recoveries and better reproducibility for a wider range of pesticides than SFE, but ASE extracts required additional steps before analysis.

Capillary Electrophoresis. An ATI Model 300 capillary electrophoresis (CE) instrument with high sensitivity optical UV detection was obtained in November of 1995. A CE method was developed for the analysis of multiple herbicides from different classesof compounds. The herbicides consisted of sulfonyl ureas (thifensulfuron-methyl, chlorimuron-ethyl, and chlorsulfuron), an imidazolinone (imazaquin), and miscellaneous others (2,4-D, acifluorfen, and bentazon). The detection limits in water were < 20 ng/mL at 240 nm for ≅ 75 nL injection volume. The running buffer, 50 mM ammonium acetate at pH 4.75, is compatible with coupling CE with electrospray ionization/MS analysis which could be used for confirmation. Investigations involving SFE, ASE, liquid-liquid partitioning, solid-phase extraction, semi-preparative and analytical HPLC, and CE were performed, and a single method was developed that achieved > 80% recoveries for herbicides in soybeans. This final method used ASE extraction and CE analysis.

Determining lipophilic pyrethroids and chlorinated hydrocarbons in fortified ground beef using ion-trap mass spectrometry. A rapid procedure has been developed that is offered as an improvement for the analysis of lipophilic insecticides in meat. This research provides a significant improvement in the multiresidue method for monitoring lipophilic insecticides in meat. The number of physical and chemical manipulations required of prior methodologies to separate analyte from fat has been minimized and the number of solvent evaporations reduced to one. This should aid in registration and reregistration activities provided by industry and various state and federal government laboratories. This research continues to assure the consumer a safe food supply by providing more rapid quantitation of samples containing insecticides and increasing the number of samples monitored. It becomes clear that as new improvements in ion-trap monitoring devices are introduced achieving low-femtogram-level sensitivity in both electron ionization and selected ion monitoring modes, the levels of sensitivity and productivity will increase providing still easier identification of trace compounds in complex matrices thus leading to further developments providing more rapid and efficient methodologies for chemical and insecticide analysis.

IMPACT/TECH TRANSFER A: An SFE pilot study to begin implementation of SFE for multiresidue analysis of pesticides in the Pesticide Data Program was initiated. The pilot study involved Departments of Agriculture for the states of California, Michigan, North Carolina, and Texas. The pilot study was designed to perform side-by-side comparison of results of samples analyzed by SFE and the traditional approach for > 50 pesticides in apple, green bean, orange, and tomato. ARS scientist, Steve Lehotay, developed the method, helped design the pilot study, wrote standard operating procedures, trained the chemists involved, and served as a consultant to answer questions

and solve problems that arose. Thus far, the method has passed rigorous validation criteria in 2 states and side-by-side analyses have begun in those states.

The method developed for herbicides in soybeans was transferred to USDA, Grain Inspection Packers and Stock Yard Administration (GIPSA), Kansas City, MO, for use in their programs.

PUBLICATIONS:

Argauer, R. J., K. I. Eller, M. A. Ibrahim, and R. T. Brown. 1995. Determining propoxur and other carbamates in meat using HPLC-fluorescence and gas chromatography/ion trap mass spectrometry after supercritical fluid extraction. *J. Agric. Food Chem.* **43**:2774–2778.

Argauer, R. J., K. I. Eller, R. M. Pfeil and R. T. Brown. 1996. Determining ten synthetic pyrethroids in lettuce and ground meat using ion-trap mass spectrometry and electron capture gas chromatography. *J. Agric. Food Chem.* (In press).

Lehotay, S. J. and A. Valverde-García. 1996. Evaluation of different solid-phase traps for automated collection and clean-up of multiple pesticides in fruits and vegetables afer supercritical fluid extration. *J. Chromatogr.* (In press).

APPLICATION OF SUPERCRITICAL FLUID TECHNIQUES TO FOOD SAFETY AND NUTRIENT ANALYSIS

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CRIS Number: **3620–42000–013**
FSIS Number: **I-2**
CRIS Completion Date: **12–17–98**

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OBJECTIVE A: Develop supercritical fluid- and conventional liquid-based size exclusion sample cleanup methods.

PROGRESS A: Previous efforts have focused on the separation of pesticides from fat using supercritical carbon dioxide modified with different organic solvents (ethyl acetate *p*-dioxane, tetrahydrofuran and *t*-butyl ether) using semi-preparative (250 mm x 10 mm) Jordi Gel "size exclusion" columns. This past year we have focused on the use of acetone as a possible modifier. Studies were initiated involving the collection of discrete fractions from the Jordi Gel columns with subsequent assaying of fat via gravimetry followed by pesticide residue analysis using GC/ECD on each fraction. Studies also were performed using ethyl acetate/cyclohexane mixtures (no carbon dioxide utilized) for comparison purposes. In all of the described studies, fat (butter) was 100% recovered at 0.5–1.0 g loadings on the Jordi Gel columns. Also, a pesticide mixture provided by the FSIS Western Laboratory yielded nearly 100% recovery on all individual pesticides. It was found that if *p*-dioxane was used as a modifier, some of the pesticide moieties decomposed upon concentrating to the final sample volume for GC/ECD analysis. In addition, overlap problems occurred between the pesticide-laden fractions and the butter fraction using the above eluents. For example, more than 70% of the butter coeluted with some of the target pesticides when an acetone/carbon dioxide (3:2) mobile phase was used, even at fat (butter) loadings of 0.1 g. The use of two Jordi Gel columns in series did not alleviate the capacity problem, but only resulted in increased retention volume, and subsequently, the volume of solvent used. For this reason, the modified "size exclusion" method employing organic solvent-modifed carbon dioxide eluents was abandoned. It should be noted that similar studies by Marvin Hopper of FDA's Total Diet and Pesticide Research Center (TDPRC) (Lexena, Kansas) using a C-1 silica-based column and a Jordi Gel column have proven very effective in separating many target pesticide analytes from fat and are the subject of a future publication by this researcher.

IMPACT/TECH TRANSFER A: Collaboration with Marvin Hopper of FDA's TDPRC has helped him develop a supercritical fluid-based cleanup scheme for separating coextracted fat from pesticide analytes.

OBJECTIVE B: Develop SF-based sample cleanup methods using binary fluids.

PROGRESS B: Studies initiated to understand the reduction in solubility experienced with using helium headspace carbon dioxide, indicated the possible application of this principle for reducing the amount of lipid coextractives experienced during SFE. Very precise density measurements on helium-entrained carbon dioxide fluid source mixtures were initiated to better understand the basic physicochemical principle behind this phenomena and how it could be exploited for reducing the amount of lipid coextractives experienced during SFE. These experiments showed that the density of helium-entrained carbon dioxide is 2–5% lower than that for neat carbon dioxide, depending on the pressure and temperature utilized.

Using a binary gas mixture of carbon dioxide/nitrogen (75 mole%/25 mole%), we have precisely measured the solubility of a typical lipid, soybean oil, in the above binary fluid. The carbon dioxide/nitrogen extraction fluid mixture reduces the solubility of soybean oil over 97% at 8000 psi and 70 °C. Although this was substantially less then experienced with pure carbon dioxide, the residual extracted lipid was still deleterious for injection onto a GC column. Using the above extraction conditions, a partially deactivated neutral alumina column (deactivated according to FSIS protocol) was inserted into the extraction cell to further reduce the lipid content of the resultant extract (to less than 1%) after passage of 300 g of the binary extraction fluid mixture. This eliminated the need for any further sample cleanup and has allowed organochlorine pesticide recoveries to be attained at 60–90% levels. Further studies are currently underway to improve these pesticide recovery levels.

IMPACT/TECH TRANSFER B: We are collaborating with Scott Speciality Gases in Plumsteadville, PA to test the extraction potential of using binary fluids in analytical SFE.

OBJECTIVE C: Develop SFE methods for mycotoxin determination in biological tissue and grains.

PROGRESS C: Methods have and are now being developed for the determination of aflatoxin M_1 in bovine liver and vomitoxin (DON) in various grains. Significant reductions have been achieved in solvent useage by combining SFE with organic cosolvents for the successful extraction of 0.3 ppb levels of aflatoxin M_1 from a bovine liver matrix. This methodology has recently been submitted to the *Journal of Food Protection* for potential publication.

A similar approach involving supercritical carbon dioxide and cosolvents also are being used for DON determination in wheat and oats by a visiting scientist. Final determination of the analyte is achieved using either a HPLC-UV method or an ELISA-based assay. The HPLC-UV method has required the use of a Vicam affinity column for sample cleanup due to the complexity of the derived extract.

IMPACT/TECH TRANSFER C: Research and method development for DON is part of the Ph.D. training of E. Jarvenpaa, a visiting scientist from the University of Turku in Finland.

PUBLICATIONS:

King, J. W., J. H. Johnson, S. L. Taylor, W. L. Orton, and M. L. Hopper. 1995. Simultaneous multi-sample supercritical fluid extraction of food products for lipids and pesticide residue analysis. *J. Supercrit. Fluids.* **8**:167–175.

King, J. W. 1995. Analytical-process supercritical fluid extraction: A synergistic combination for solving analytical and laboratory scale problems. *Trends Anal. Chem.* **14**:474–481.

King, J. W. and K. Nam. 1996. Coupled enzyme immunoassay (EIA) with supercritical fluid extraction (SFE), pp 423–438. *In* Immunoassays for Residue Analysis: Food Safety, R. C. Beier and L. Stanker (eds), ACS Symposium Series 621, American Chemical Society, Washington, DC, 528 pp.

Final Report: BIOSENSORS IMMUNOCHEMICAL DETECTION METHODS FOR RESIDUES

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CRIS Number: **5325–42520–001**
FSIS Number: **I-2**
CRIS Completion Date: **8–01–96**

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OBJECTIVE A: Apply immunochemical and biosensor methods to quantify components of foods which influence the safety and healthfulness of the food supply.

PROGRESS A: This research builds on the previous work done to develop screening assays for benzimidazole anthelmintics, including thiabendazole, fenbendazole, oxfendazole, oxibendazole, cambendazole, and albendazole. A Cooperative Agreement, initiated in November 1994 with Millipore Corporation (Bedford, MA), facilitated commercialization of our thiabendazole method as part of the EnviroGard immunoassay kit product line, which was acquired in March 1996 by EnSys, Inc. (Research Triangle Park, NC). A complete protocol for the multi-residue benzimidazole assay was submitted to FSIS Chemistry Division (May 1996). The protocol will permit evaluation of the two assay systems, thiazole and 2-methylcarbamates. Work on patterning immobilized antibody layers via photolithography and oxygen plasma exposure was completed, with fluorescent antibody studies demonstrating resolution of line widths of 2–3 micrometers. The results provide the basis for fabrication of multianalyte sensors.

IMPACT/TECH TRANSFER A: An immunoassay kit manufactured by EnSys, Inc. is commercially available to FSIS and producers, enabling measurement of residues of thiabendazole, 5-hydroxythiabendazole, and cambendazole. Immunochemical methods for 2-benzimidazole methyl carbamates have been completely documented in a protocol presented to FSIS.

PUBLICATIONS:

Brandon, D. L., A. W. Flounders, R. G. Binder, A. H. Bates, W. C. Montague, Jr., and R. Jackman. 1995. Development of antibodies and antibody immobilization procedures for optimized benzimidazole-specific immunoassays and immunosensors, Abstract AGRO-0020. International Chemical Congress of the Pacific Basin Societies, Honolulu, HI.

DISPOSITION OF BETA-AGONISTS IN FARM ANIMALS

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CRIS Number: **5442–32000–006**
FSIS Number: **I-90–6**
CRIS Completion date: **2–01–97**

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OBJECTIVE A: Determine the metabolism, distribution, excretion, and elimination properties of β-adrenergic agonists in food producing animals.

PROGRESS A: A previous report described the distribution of the total radioactive residues administered as ^{14}C-clenbuterol in Holstein calves. In order to determine the relationship between parent clenbuterol and the total radioactive residues, parent clenbuterol was measured in heart, lung, liver, kidney, and spleen. Parent clenbuterol present in skeletal muscle is not reported due to the very low recoveries encountered during the assay. Parent clenbuterol comprised 43.9, 62.9, 62.8, 57.7, and 81.9% of the total radioactivity present in liver, kidney, heart, spleen (one animal only) and lungs. The chiral composition of the clenbuterol residues remaining in tissues and present in urine is being determined.

A study designed to determine the effect of the ^{14}C-clenbuterol dose and withdrawal period on the total radioactive residues in tissues of broiler chicks has been conducted. Broiler chickens, 5 weeks of age, were given dietary ^{14}C-clenbuterol•HCl (0.5, 1.0, or 2.0 ppm) for 14 consecutive days. Four birds from each dietary clenbuterol level were slaughtered at a withdrawal period of 0, 7, and 14 days. At slaughter, 14 tissues were collected, processed, and analyzed for total radioactivity. Selected tissues will be assayed for the concentration of parent clenbuterol; when practical, the chiral composition of the clenbuterol residue also will be assessed.

IMPACT/TECH TRANSFER A: These studies have shown that parent clenbuterol represented less than one-half of the total residues after ^{14}C-clenbuterol administration to calves. Because inadvertent human intoxication by clenbuterol has occurred after the consumption of liver from illegally treated calves, the contribution of metabolites to clenbuterol poisoning should be investigated.

PUBLICATIONS:

Smith, D. J. and G. D. Paulson. Distribution, elimination, and residues of [^{14}C]clenbuterol HCl in Holstein calves. *J. Anim. Sci.* (In press).

DIOXINS IN BEEF, MILK, AND FORAGE

ARS Contact Persons:
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CRIS Number: **5442–42000–001**
CRIS Completion Date: **9–01–98**

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OBJECTIVE A: Identify and quantify residues of chlorinated dioxins and furans in beef and in animal feeds, in particular, forages. Devise strategies for minimizing the occurrence of these residues in meat products.

PROGRESS A: The United States Environmental Protection Agency (EPA) has designated beef as a major contributor to the human dioxin burden because animals grazing on forage contaminated by fallout from burning processes would store these lipophilic materials in adipose tissue. FSIS also is very interested in dioxin levels in beef because of their food safety concerns. The study described here was designed to provide data on the dioxin burden that the population receives from the consumption of beef. Analyses of domestic beef samples in a study by FSIS/EPA and in our geographic survey generally showed low concentrations of dioxins and furans (often at a non-detectable level). However, some animals had concentrations that were many times greater than others at a given site. Also, the control animals in a feeding study conducted at Carrington, ND had concentrations of some of the higher chlorinated congeners that were equal to those of the experimental animals, indicating a source of contamination that equaled or exceeded our feeding amounts. Analyses of components in the feeding facilities strongly suggest that the major source of dioxins in animals with high levels is wood (i.e. support posts and dividing planks) treated with pentachlorophenol. Analyses of components from feeding facilities from two sites that produced animals with the greatest concentrations (Oregon State University and Penn State University), also had posts and planks with high concentrations. All of these facilities had been built in the early 1970s. The animals from our feeding study yielded samples having dioxin concentrations sufficiently above the non detect level to determine the distribution of dioxin and furan congeners among several lipid containing matrixes (serum, back fat, perirenal fat, rib eye and liver). On a weight basis, back fat and perirenal fat have the highest levels of the lower chlorinated congeners while liver has the highest levels of the higher chlorinated congeners. On a lipid adjusted basis the tetra isomers are evenly distributed (within a factor of two) among the tissues analyzed, whereas the higher chlorinated isomers are found predominantly in the liver. Back fat and perirenal fat appear to be equally suitable tissues for monitoring levels of the lower chlorinated congeners with perirenal fat being the more suitable for lean animals where back fat is often scarce or nonexistent.

IMPACT/TECH TRANSFER A: The use of pentachlorophenol treated wood in construction of animal feeding facilities appears to be a major source of dioxins in U.S. beef. Liver appears to be the most sensitive measure for levels of the high chlorinated congeners, although the levels found in back fat and perirenal fat also are sufficient for analysis.

OBJECTIVE B: Study the metabolic disposition and excretion of dioxins in animals so that it can be used to help minimize the occurrence of residues in mammalian tissue metabolism.

PROGRESS B: Metabolism studies of 1,2,7,8-, 1,3,7,8-, and 1,4,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which are considered to be less toxic dioxin isomers, have been conducted in rats and bile-cannulated rats and calves. Rats were dosed orally with one of these ^{14}C-labelled TCDD congeners at a level of 7–8 mg/kg. Urine, bile, and feces were collected over 24 hr periods for 3 days. Over 95.1, 99.7 and 94.7% of the dose from these three TCDD congeners, respectively, was excreted within 3 days. The major route of excretion was rat feces where 79.8, 93.8 and 88.8% of the ^{14}C of the dose from these three TCDD congeners, respectively, was excreted within 3 days. In the bile-cannulated rats, 26.8, 29.7 and 33% of the ^{14}C was found in the first 24 hr bile collection indicating significant absorption of these three congeners, respectively. The liver was the only organ which appeared to have significant ^{14}C residues from these three TCDD congeners. Major metabolites isolated and identified from 1,2,7,8-TCDD dosed rats or calf were: 2-O-glucuronide-1,3,7,8-TCDD (rat bile), 2-OH-1,3,7,8-TCDD (rat feces and calf feces), and the sulfate ester of 4,5-dichlorocatechol (rat urine). In 1,3,7,8-TCDD dosed rats, the two major fecal metabolites were identified as 2-OH-1,4,7,8-TCDD and 3-OH-1,2,7,8-TCDD. The major metabolites isolated and identified from the 1,4,7,8-TCDD dosed rats were 2-OH-1,4,7,8-TCDD, 1-OH-4,7,8-TriCDD, the glucuronide conjugates of these phenols, and a dihydroxytetrachlorodioxin conjugated with both a glucuronide and a sulfate ester. These oxidized metabolites were formed via arene oxides and subsequent conjugation. A minor metabolite, 4,5-dichlorocatechol, was formed by cleavage of the dioxin ring which may be initiated by arene oxide formation at the bridging carbons. Initially, no parent compound was observed in the feces, but after some time (one week of standing) the parent was found and it accounted for 6% of the dose. This suggests the presence of an unstable metabolite which reverts back to parent. Although this hypothesis has not yet been proven for dioxins, previous studies have shown that certain arene oxide metabolites can revert back to parent aromatics (V. J. Feil *et al.* 1986. Polynuclear Aromatic Hydrocarbons: Chemistry, Characterization and Carcinogenesis, pp 263–269. Ninth International Symposium, Batelle Press, Columbus, OH). It also was observed that another common metabolic pathway for chlorinated aromatics, the mecapturic acid pathway, did not play a role in the metabolism of dioxins.

IMPACT/TECH TRANSFER B: These results show that although 1,2,7,8-, 1,3,7,8-, and 1,4,7,8-TCDD are rapidly excreted, residues do remain in the liver after 72 hrs. Over 27–30% of the doses of these three TCDD congeners were absorbed by the animal as

shown by the biliary excretion data. The metabolites isolated indicate that a major route of metabolism is the arene oxide pathway for these TCDD congeners. Some evidence suggests that there is formation of unstable metabolites that revert back to the parent and could lead to persistance of the parent dioxin in the animal.

PUBLICATIONS:

Feil, V. J., K. L. Davison, T. O. Tiernan, and V. L. Anderson. 1996. Distribution of polychlorinated dioxins and furans in beef. *Organohalogen Comp.* **28**:152–155.

Fries, G. F., F. J. Feil, and K. L. Davison. 1996. The significance of pentachlorophenol-treated wood as a source of dioxin residues in United States beef. *Organohalogen Comp.* **28**:156–159.

Huwe, J. K., V. J. Feil, and G. L. Larsen. 1996. Identification of the major metabolites of 1,4,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Organohalogen Comp.* **29**:462–467.

Larsen, G. L., V. J. Feil, H. Hakk, J. K. Huwe, and E. Petroske. 1996. Polychlorodibenzo-*p*-dioxin metabolism. *Organohalogen Comp.* **28**:491–494.

PHARMACOKINETIC MODELS FOR PERSISTENT TOXIC CHEMICALS IN FARM ANIMALS

ARS Contact Persons:
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CRIS Number: **1265–32000–046**
FSIS Number: **I-89–1**
CRIS Completion Date: **9–01–97**

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OBJECTIVE A: Develop mathematical models of the pharmacokinetics of TCDD and related compounds in beef and dairy animals.

PROGRESS A: A review of the EPA-FSIS survey of the U.S. beef supply and the ARS study of 12 institutional facilities revealed that the chlorinated dibenzo-*p*-dioxin (CDD) and dibenzofuran (CDF) congeners that occurred most frequently and with the highest mean concentrations were 1,2,3,6,7,8-CDD, 1,2,3,4,6,7,8-CDD, and 1,2,3,4,6,7,8,9-CDD. These congeners are typical of the contaminants found in pentachlorophenol (PCP); whereas, residues of combustion are dominated by CDFs. Environmental samples were collected at several locations in the ARS study where the beef had higher than average concentrations. One or more wood samples with high concentrations of CDDs were identified at each location. The congener profiles in the beef fat samples at these locations were comparible with the profiles in the wood samples adjusted for differential absorption by animals. No other feed or environmental sample contained sufficient CDD residues to account for the level of animal residues. The use of PCP-treated wood for animal housing and confinement facilities, the propensity of animals to lick or chew on wood, and the comparibility of congener profiles lead to the conclusion that PCP-treated wood is an important source of CDDs and CDFs in animal products.

Stable concentrations of CDDs and CDFs in milk were reached within 28 days when lactating cows were administered PCP-treated wood. CDFs without chlorine substitution in the 4 or 6 positions were not accumulated in milk or tissue. Accumulation of other CDFs and CDDs were related inversely to the degree of chlorination. Residues of 1,2,3,6,7,8-CDD, 1,2,3,4,6,7,8-CDD, and 1,2,3,4,6,7,8,9-CDD tended to become more dominate as time increased. Quantities of octa- and hepta-CDDs in excess of the administered dose were recovered in the feces. The most logical explanation for the excess is synthesis by rumen fermentation. Synthesis also has been reported in composting and sewage treatment. The relative roles of potential precursors must be established. If the mechanism is confirmed, it may be inferred that the two congeners probably are synthesized in other anaerobic environments.

Coplaner PCBs (polychlorinated biphenyls) are compounds with dioxin-like activity that are less well characterized than the dioxins and furans. Cooperative research with the Ohio Agricultural Research and Development Center is utilizing archived samples and data from a dairy cow experiment with the Aroclor 1254 PCB mixture. Samples of milk, adipose tissue, plasma and feces are being re-analyzed on an individual congener basis.

IMPACT/TECH TRANSFER A: The impact of the findings is uncertain pending more complete characterization.

PUBLICATIONS:

Fries, G. F. 1996. A model to predict concentrations of lipophilic compounds in growing animals. *Chemosphere* **32**:443–451.

Fries, G. F. 1996. Ingestion of sludge applied organic chemicals by animals. *Sci. Total Environ.* **185**:93–108.

PREVENTION OF MYCOTOXICOSIS IN POULTRY AND LIVESTOCK

ARS Contact Persons:
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CRIS Number: **6202–32000–008**
CRIS Completion Date: **9–01–97**

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OBJECTIVE A: Determine the efficacy of selected adsorbents to reduce the toxicity of mycotoxins in poultry and livestock.

PROGRESS A: Research was continued on determining the efficacy *in vivo* of adsorbents with the ability to adsorb mycotoxins *in vitro*. Recent results from experiments have shown that a super-activated charcoal, as-well-as a hydrated sodium calcium aluminosilicate (HSCAS), were effective in reducing the toxicity of aflatoxins from rice powder culture material, when added to the diets of broiler chicks. Another aluminosilicate product was not effective in protecting against the toxicity of aflatoxins (AFs). These results further emphasize the fact that all silicate-type sorbents are not equal in their ability to protect against the toxicity of aflatoxins. None of the sorbents studied to date have demonstrated the ability to reduce the toxicity of T-2 toxin or any of the other mycotoxins that might be consumed by animals.

A HSCAS, acidic HSCAS, and activated charcoal (AC) administered concomitantly with aflatoxin B_1 (AFB_1) to previously colostomized turkey poults were shown to adsorb AFB_1, thereby limiting AFB_1 bioavailability and reducing urinary excretion of aflatoxin M_1 (AFM_1). Importantly, the addition of HSCAS, acidic HSCAS, or AC did not modify the metabolic profile of AFB_1. In this experiment, activated charcoal reduced AFM_1 output but failed to alleviate toxicosis when included in AF-contaminated diets fed to growing turkey poults or in previous experiments with growing chicks. These findings suggest that protection by AC may occur via nonspecific binding of aflatoxin B_1 and this binding may be altered in the presence of competing ligands.

IMPACT/TECH TRANSFER A: These results indicate that the addition of some dietary adsorbents can alleviate some of the toxic effects associated with aflatoxin without modifying the metabolic profile of aflatoxin B_1, and without causing the formation of new metabolites. The effectiveness and limitations of this technology have been transferred through publications in scientific and trade journals and numerous contacts with personnel in poultry, livestock, grain producers, and allied industries and universities throughout the U.S. and abroad.

OBJECTIVE B: Determine the toxicity of fumonisins and other mycotoxins and the potential interactions between these and other mycotoxins and chemicals in poultry and livestock.

PROGRESS B: Investigations to determine and characterize the toxicity of mycotoxins individually and in combination continued. Experiments involving *Fusarium moniliforme* (M-1325) culture material (FMCM) containing fumonisins, aflatoxins, diacetoxyscirpenol, ochratoxin A, T-2 toxin, or deoxynivalenol-contaminated wheat or *Fusarium fujikuroi* culture material containing moniliformin and aflatoxin were completed in broiler chicks and turkey poults. Experiments were conducted with swine fed FMCM and aflatoxin or deoxynivalenol and with lambs fed FMCM. Fortunately for producers, concentrations of 200 ppm or higher of fumonisins were necessary in poultry diets before performance was adversely affected. Although, toxicity of the toxin combinations was more than the individual toxins, it can best be characterized as additive or less than additive. The exception was a synergistic response that caused a decrease in body weight gain and liver weights in growing swine fed diets calculated to contain 100 ppm fumonisin B_1 from *Fusarium fujikuroi* and 5 ppm deoxynivalenol from naturally contaminated wheat.

IMPACT/TECH TRANSFER B: These results indicate that concentrations of fumonisins much higher than are being found under field conditions are necessary before toxicity is observed in poultry, swine, and lambs. However, this research also demonstrates that combinations of mycotoxins are more toxic than the individual mycotoxins, and this factor should be considered when selecting ingredients and formulating diets. This technology has been transferred through scientific publications and trade journals and numerous contacts with producers, feed manufacturers, and related industries throughout the U.S. and abroad.

OBJECTIVE C: Determine the significance of dietary modifications on the expression of mycotoxin toxicity.

PROGRESS C: Investigations to further characterize the effects of dietary modifications on the toxicity of mycotoxins continue. Dietary addition of the antibiotics, lincomycin and tylosin, to growing swine diets containing aflatoxin did not alter the toxicity of aflatoxins. From these data, we do not know whether aflatoxins affect the efficacy of these compounds. Administering injectable vitamin E to growing swine fed diets containing aflatoxin did not reduce the toxicity of aflatoxin. Aflatoxin reduced body stores of vitamins A and E. Administration of vitamin E to swine receiving a diet without aflatoxins caused an increase in serum α-tocopherol concentrations. α-Tocopherol concentratons in swine cardiac tissue of the aflatoxin plus vitamin E group were increased over those of the aflatoxins alone group, indicating an ameliorating effect on tissue reductions of tocopherol that were induced by aflatoxins.

IMPACT/TECH TRANSFER C: Lincomycin and tylosin had no effect on aflatoxicosis and it is unknown if aflatoxins affect the efficacy of these antibiotics. Vitamin E did not reduce aflatoxicosis; however, aflatoxins reduced body stores of vitamins A and E, which may exacerbate vitamin deficiencies. This technology has been transferred through scientific publications and trade journals and numerous contacts with pork producers, feed manufacturers, and related industries throughout the U.S. and abroad.

PUBLICATIONS:

Edrington, T. S., C. A. Kamps-Holtzapple, R. B. Harvey, L. F. Kubena, M. H. Elissalde, and G. E. Rottinghaus. 1995. Acute hepatic and renal toxicity in lambs dosed with fumonisin-containing culture material. *J. Anim. Sci.* **73**:508–515.

Edrington, T. S., A. B. Sarr, L. F. Kubena, R. B. Harvey, and T. D. Phillips. 1996. Effects of hydrated sodium calcium aluminosilicate (HSCAS), acidic HSCAS, and activated charcoal on the metabolic profile and toxicity of aflatoxin B_1 in turkey poults. *Toxicol. Lett.* **76**: (In press).

Harvey, R. B., T. S. Edrington, L. F. Kubena, D. E. Corrier, and M. H. Elissalde. 1995. Influence of the antibiotics lincomycin and tylosin on alfatoxicosis when added to aflatoxin-contaminated diets of growing swine. *J. Vet. Diagn. Invest.* 7:374–380.

Harvey, R. B., T. S. Edrington, L. F. Kubena, M. H. Elissalde, H. H. Casper, G. E. Rottinghaus, and Turk, J. R. 1996. Effects of dietary fumonisin B_1-containing culture material, deoxynivalenol contaminated wheat, or their combinations in the diets of growing barrows. *Am. J. Vet. Res.* **57**: (In press).

Harvey, R. B., T. S. Edrington, L. F. Kubena, M. H. Elissalde, and G. E. Rottinghaus. 1995. Influence of aflatoxin and fumonisin B_1-containing culture material on growing barrows. *Am. J. Vet. Res.* **56**:1668–1672.

Kubena, L. F., T. S. Edrington, R. B. Harvey, T. D. Phillips, A. B. Sarr, and G. E. Rottinghaus. 1996. Individual and combined effects of fumonisin B_1 present in *Fusarium moniliforme* culture material and diacetoxyscirpenol or Ochratoxin A in turkey poults. *Poult. Sci.* **75**: (In press).

Kubena, L. F., T. S. Edrington, C. A. Kamps-Holtzapple, R. B. Harvey, M. H. Elissalde, and G. E. Rottinghaus. 1995. Influence of fumonisin B_1 present in *Fusarium moniliforme* culture material and T-2 toxin on turkey poults. *Poult. Sci.* **74**:306–313.

Kubena, L. F., T. S. Edrington, C. A. Kamps-Holtzapple, R. B. Harvey, M. H. Elissalde, and G. E. Rottinghaus. 1995. Effects of feeding fumonisin B_1 present in *Fusarium moniliforme* culture material and aflatoxin singly and in combination to turkey poults. *Poult. Sci.* **74**:1295–1303.

Kubena, L. F., R. B. Harvey, S. A., Buckley, T. S. Edrington, and G. E. Rottinghaus. 1996. Individual and combined effects of moniliformin present in *Fusarium fujikuroi* culture material and aflatoxin in broiler chicks. *Poult. Sci.* **75**: (In press).

Norage, M. A., T. S. Edrington, L. F. Kubena, and R. B. Harvey. 1995. Influence of a hydrated sodium calcium aluminosilicate and virginiamycin on aflatoxicosis in broiler chicks. *Poult. Sci.* **74**:626–632.

INDICATION OF TEMPERATURE TO WHICH MUSCLE MEAT PRODUCTS HAVE BEEN COOKED

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FSIS Number: **I-5**
CRIS Completion Date: **6–01–99**

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OBJECTIVE A: Determine acid phosphatase (ACP) activity in model cooked turkey thigh meat.

PROGRESS A: In a cooperative study with Dr. Denise Smith, Department of Food Science, Michigan State University, acid phosphatase (ACP) acitvity was determined in ground turkey thigh meat to determine time/temperature enzyme related activity loss. To determine if ACP activity could be used as an end point temperature indicator, ACP thermal enzyme inactivity D-values and subsequent Z-values were determined for turkey muscle heated to 4 temperatures (55, 58, 61, and 63 °C). The D- and Z-values were then compared with certain pathogenic food microrganisms. Results showed a Z-value of 6.34 °C with an R_2 of 0.9995. The D-values were 329.6, 104.0, 35.7, and 12.5 min at 55, 58, 61,and 63 °C, respectively.

IMPACT/TECH TRANSFER A: These results will allow the use of ACP activity as an end-point temperature (EPT) verification method for pathogenic food microorganisms with similar Z-values (6.34 °C) in turkey products which have EPT requirements within the tested temperature range. This method has potential for FSIS to use as a verification method for certain cooked turkey products. Further, the method is being adapted by a cooperating commercial manufacturer for a quality assurance/HACCP methodology for use by commercial poultry processors.

OBJECTIVE B: Determine myoglobin denaturation rates in cooked ground beef.

PROGRESS B: Myoglobin deaturation is under study in cooperation with HemoCue Inc., Angelholm, Sweden to determine if it can be used as an EPT indicator for cooked ground beef (hamburger) patties. The method is based on a rapid biochemical method currently in use to measure hemoglobin in the medical field. HemoCue has made some biochemical changes to optimize their test for myoglobin. In a cooperative study wtih Dr. Anna Reserection and Kay McWaters, Center for Food Safety and Quality Enhancement, University of Georgia, the myoglobin test showed a significant difference between patties cooked to 68.3 and 71.1 °C and among three treatment groups: 1) Control, (frozen raw,

cooked non-stored); 2) 30 days stored (frozen raw, cooked, 30 day stored frozen, reheated to 60.0 °C); and 3) 60 days stored (frozen raw, cooked, 60 day stored frozen, reheated to 60.0 °C). These results show that this 5 min test could have potential as an EPT indicator for verifying FSIS/FDA requirements for fully cooked hamburger patties.

IMPACT/TECH TRANSFER B: Current research findings show this method could be used by the FSIS Food Chemisty lab, state or local health departments to verify compliance of beef pattie products having been heat processed to 155 °F or 160 °F required by the Pattie product regulation. Further, the method could be used in fast food quality assurence programs to verify compliance with FSIS/FDA regulations.

OBJECTIVE C: Determine the potential of residual glutamic-oxaloacetic transaminase (GOT) activity in thermally processed beef and poultry as an indicator of EPT's.

PROGRESS C: We have cooperated with poultry processing companies to investigate an incident that concerned thigh meat that had the appearance of being undercooked, and another that involved undercooking breast meat. In both instances, rejection of the product by the retailer had occurred. The products were analyzed for residual glutamic-oxalacetic transaminase (GOT) activity which was subsequently compared with data derived in our laboratory. The thigh meat samples had been cooked adequately to the required 71.1 °C, yet retained an uncooked appearance (red color for meat and juice). Subsequent research revealed that the anterior adductor muscles (darker colored thigh meat) retained red coloration of juices after cooking to 70, 72 and 75 °C EPT's. Analysis of the breast patties for residual GOT activity indicated inadequate cooking. Residual GOT activity in the samples was too high to estimate the EPT that had been achieved; however, it was less than 71.1 °C required for safe consumption. Recommendations were made to review cooking and quality-control procedures. Studies to refine analytics for measuring residual GOT activity in cooked poultry and beef samples and the subsequent use of procedures to estimate EPT's of commercial samples continue.

IMPACT/TECH TRANSFER C: Results for commercial samples indicate the potential of these procedures as part of a HACCP program to accurately estimate EPT's in cooked poultry and beef products.

OBJECTIVE D: Color stability of beef juices during storage.

PROGRESS D: The stability of the residual red/pink color of beef juices was determined immediately after cooking of top-round samples to selected EPT's and then after storage of the intact tissue at –20 °C for 3 weeks. Also, stability of color was determined during storage of the expressed juices at 3 °C under N_2 for up to 72 hrs. Data showed that freezing of the cooked, intact tissue caused no significant change in the red color of juices from samples cooked to 76, 78, 80, 82 and 84 °C. Storage of the expressed juices in the

inert atmosphere resulted in loss of color. Intensity of redness decreased significantly within 24 hrs.

IMPACT/TECH TRANSFER D: Freezing the imported cooked meats from South America causes no significant changes in the red/pink color of the juices that are evaluated in the "pink-juice" test; however, evaluations by FSIS inspectors must be made immediately after the juices are expressed from the meat.

OBJECTIVE E: Evaluate factors which affect cooked poultry meat color.

PROGRESS E: Color is frequently used to assess doneness of meat. However, color as assessed subjectively is not always an accurate method for determining doneness because undercooked meat can appear cooked and fully cooked meat can appear undercooked. A study was conducted to correlate objectively measured color of cooked meat with a biochemical indicator of doneness. Another study evaluated effects of processing variables on cooked meat color. In the first study, chicken patties were cooked to EPT's of 52.9 to 88.8 °C and then evaluated for CIE color values and GOT activity. Both measures were negatively correlated with EPT (–0.74 for color values and –0.88 for GOT activity), however, neither precisely estimated EPT. In the second study, chicken front quarters were marinated in solutions containing polyphosphates (compounds routinely used in further processed poultry products to improve moisture binding properties) for various post-chill times ranging from 0 to 6 hrs. As post-chill aging time increased prior to polyphosphate incorporation, redness of the cooked meat (a values) decreased.

IMPACT/TECH TRANSFER E: The response of broiler breast muscles over post-chill aging times to differences in redness for the cooked meat indicates the limitations of subjective color evaluations to determine adequate cooking. This information is important to both FSIS inspectors and poultry processors.

PUBLICATIONS:

Senter, S. D., G. K. Searcy, and L. L. Young. 1996. Glutamic-oxaloacetic transaminase (GOT) activity in commercially processed chicken: an indicator of product end-point temperature. *J. Food Prot.* (In press).

Young, L. L. and S. D. Senter. 1996. Evaluation of meat color and biochemical indicators of endpoint cooking temperature of chicken meat patties. Proceedings of the World's Poultry Science Association, New Delhi, India. (In press).

IDENTIFYING AND CONTROLLING MECHANISMS ASSOCIATED WITH QUALITY OF REDUCED FAT MEAT PRODUCTS

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OBJECTIVE A: Identify mechanisms and develop control procedures to prevent inconsistent cooked meat color/cooked meat temperature relationships.

PROGRESS A: A number of studies were conducted to determine the effects of formulation, processing, storage conditions and cooking procedures on cooked internal color and temperature variability in beef patties. The following factors in processing patties were frequently associated with the appearance of visual pink/red color when patties were cooked to 71 °C: (1) cow meat, (2) high pH muscle (above 6.0), (3) inclusion of oat products, and (4) cooking from the frozen state. Cooking of frozen patties to internal temperatures between 81 and 87 °C was necessary for complete disappearance of pink/red color in some formulations. Premature browning in patties cooked to 71 °C was occasionally observed due to: (1) high metmyoglobin formation, (2) thawing patties prior to cooking, (3) use of meat from advanced recovery systems, and (4) inclusion of soy and whey protein products. Patties which displayed substantial pink/red color when cooked to 71 °C from the frozen state would often appear brown when cooked to 71 °C after 24 hrs of thawing at 7 °C. The effects of thawing on the development of premature browning appear independent of those exerted by metmyoglobin formation. These results indicate that internal color of ground beef patties is not a reliable indicator that the product has reached a specific endpoint temperature. In studies designed to document the variability in cooking properties of beef patties when considerable control was exerted on cooking procedures, wide variation in cooking time was noted when patties were cooked to a constant internal temperature. Conversely, cooking patties for a constant time period produced sizable variation in internal temperature. The typical range in internal temperature among patties was 68 to 75 °C, for patties cooked for a constant time to 71 °C on the same platters in a convection oven. Studies have been performed to ascertain the degree of temperature variability within patties during cooking. Internal temperature of ground beef patties at three locations (center, 1/3 and 1/6 of patty diameter) was monitored using thermocouples during the final stages of cooking to 71 °C. Temperature curves differed substantially regardless of the thermocouple location, ground beef formulation or replicates within a formulation. At the end of heating, 58.33% of the patties had a maximum temperature difference between the three locations of 5.5 °C or

less, while a temperature difference greater than 11.1 °C occurred in 4.17% of the patties. This clearly demonstrates that more complete determination of internal temperature within patties is necessary to assure safety.

IMPACT/TECH TRANSFER A: Meetings were held with officials of both FSIS and AMS regarding cooked color problems in ground beef. Assisted FSIS with consumer focus groups designed to provide input from consumers on what they consider to be "well-done" cooked ground beef. Provided input and review to FSIS on new published technical information which stressed that meat temperature and not color should be the major method used by consumers to determine doneness. On numerous occasions, provided written materials to various ground beef processors, FSIS, Australian government, public schools, public health agencies and consumers regarding color issues in ground beef.

PUBLICATIONS:

van Laack, R.L.J.M., B. W. Berry, and M. B. Solomon. 1996. Variations in internal color of cooked beef patties. *J. Food Sci.* **61**:410–414.

Final Report: LIPID OXIDATION PRODUCTS IN FRYING OILS AND FOODS AS POTENTIAL HEALTH HAZARDS

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CRIS Number: **5325–42000–014**
FSIS Number: **I-93–3**
CRIS Completion Date: **12–01–96**

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OBJECTIVE A: Study the effect of frying variables such as frying temperature and oil type on the possible production of mutagenic substances in repeatedly used frying oils while monitoring oil quality with the AOCS official methods.

PROGRESS A: Seven commonly used frying fats and oils (beef tallow, canola oil, partially hydrogenated canola oil, corn oil, cottonseed oil, soybean oil, and partially hydrogenated soybean oil) were heated at two different temperatures (190 and 204 °C) for eight hrs/day until they reached a critical level of polar constituents (25% is the limit in several European countries). The fatty acid composition of each original oil was determined by gas chromatography (GC). Besides the polar compounds, the levels of dimeric and polymerized triglycerides (DPTG), iodine value (IV) and color index (CI) were analyzed daily.

The results of this investigation clearly showed that oils deteriorate faster at higher temperatures and that the rate of deterioration is different for each oil. In general, oils with higher levels of unsaturated fatty acids produced more polar compounds compared to the more saturated ones. The fatty acid composition, especially the content of linolenic acid (18:3), also played an important role. For example, soybean oil had the highest iodine value (IV - 131) of the oils under investigation (i.e., the highest degree of unsaturation) though corn (IV - 126) and cottonseed (IV - 114.5) oil contained more linolenic acid. Our studies showed that these latter two oils also formed more polar compounds than did soybean oil. The formation of polar compounds was correlated to the temperature. When heated at 204 °C cottonseed oil yielded the highest amount of polar material while at 190 °C corn oil produced the most polar material. Beef tallow, the most saturated of the frying media (IV - 46), had the lowest yield of polar material.

Since the analysis of polar compounds is commonly referred to as the most accurate method for measuring frying oil degradation, we calculated its correlation to the other methods employed. Analysis of DPTG by high performance liquid chromatography (HPLC) with gel permeation columns showed the best correlation (correlation factor of 0.997) within the entire range of oils; whereas, the other methods (iodine value and color

index) showed mixed results. Their mean correlation value was 0.984 for the iodine values and 0.975 for the color index.

Mutagenicity of the oils was tested using the Ames assay. We tested polar fractions of the oils with five different *Salmonella* tester strains (TA 97, TA 98, TA 100, TA 102 and TA 104), with TA 97 and TA 104 being the most widely used. Oils were tested at their heating endpoint which also showed the highest level of polar material. The polar and non-polar fractions of all oils were tested with TA 97. The polar fractions of all oils were tested with TA 104. Canola oil and soybean oil were tested daily to examine the effect of heating time. We have not detected any mutagenic activity in any of the polar and non-polar oil fractions with the Ames assay. Volatile fractions of the oils (obtained by simultaneous distillation-extraction (SDE)) were tested for mutagenic actvity with a modified liquid preincubation method and *Salmonella* strain TA 104. No mutagenic activity was found in these samples.

IMPACT/TECH TRANSFER A: No mutagens were found in frying oils containing maximum allowable levels of polar constituents. This finding should reduce public concern about the potential for mutagenicity in repeatedly used frying oils.

PUBLICATIONS:

Takeoka, G. R., C. Perrino, and R. G. Buttery. 1996. Volatile constituents of used frying oils. *J. Agric. Food Chem.* 44:654–660.

APPLICATION OF SUPERCRITICAL FLUID TECHNIQUES TO FOOD SAFETY AND NUTRIENT ANALYSIS (fat analysis)

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OBJECTIVE A: Conduct a collaborative study to show equivalence of supercritical fluid extraction (SFE)-based method to the Nutritional Labeling and Education Act (NLEA) and similar protocols for fat analysis.

PROGRESS A: The collaborative study was initiated over this past year with 9 different laboratories participating. Initially it was anticipated that NCAUR would run all the required GC-FAME analysis due to our inability to locate nine laboratories which could, or would be willing to perform both extraction via SFE, and the required analysis mandated by NLEA. However, after consultation with AOAC and David Soderberg of FSIS, it was decided that the GC-FAME analysis would have to be done at a number of laboratories to have a truly valid study. The required cooperating laboratories were finally located, and the nine types of meat samples (18 samples as blind duplicates) were sent to the laboratories agreeing to participate in the study. All of the extracted samples were collected in Peoria for GC-FAME analysis and these were sent to the requisite number of laboratories for GC-FAME analysis. As of September 3, 1996, four of the nine analysis laboratories have completed their analysis, and the data received so far is not encouraging. Despite encouraging results at NCAUR during method development, there is high variability between laboratories and within the laboratories for a given type of meat. Possible sources of this variation appear to be incomplete retention of the fat on the Empore disk; the methanol rinse of the disk may remove some fats before extraction; incomplete extraction during SFE; incomplete transesterification of the fat; or the internal standard to FAME ratio is to low for the analysis method. We are currently studying these parameters to determine the source of error and, if the varibility problem can be solved, we may run a less extensive peer validated study on the method.

IMPACT/TECH TRANSFER A: Through running the above collaborative study we have introduced a number of laboratories to the possibility of using analytical SFE for nutrient analysis, including the FSIS participant, and the Eastern Laboratory.

OBJECTIVE B: Apply SFE and SFC for the analysis of cholesterol in meat products.

PROGRESS B: The method described for determining cholesterol in meat samples performed on a Suprex Autoprep 44 previously, was modified slightly, since the commercial unit had to be returned to Suprex after the initial loan period. Method development continued on an Isco SFX-2–10 using fluoroform to minimize the coextraction of other lipid moieties with cholesterol. The substitution of fluoroform for carbon dioxide did not prove advantagous and also resulted in appreciable amounts of lipid being coextraced. An Applied Separation SPEED unit also was utilized in recent studies employing a variety of adsorbents within the extraction cell and off-line after SFE. To date, an aminopropyl bonded material in a solid phase extraction (SPE) cartridge used after SFE has proven to be the most effective sorbent. Recoveries from fortified matrices are approaching 100 % using capillary SFC for analysis.

IMPACT/TECH TRANSFER B: We are working with Applied Separations, Allentown, PA on the integration of SPE cartridges and materials into the SFE method.

OBJECTIVE C: Develop an enzymatic based hydrolysis/transesterification procedure for use in NLEA fat analysis.

PROGRESS C: Additional studies were performed to optimize the previously reported, totally automated SFE method, for fat analysis in meats. Initially the effect of moisture in the meat samples was found to limit the degree of transesterification achieved via lipase catalysis, but the effect of moisture could be minimized by freeze drying the samples prior to SFE. Conversions to the requisite fatty acid methyl esters (FAMEs) was found to be over 99% for all extraction/reactions run on ground beef, sausage, and oilseed samples, and the total fat content was found to agree with those determined via traditional solvent extraction methods. The conversion and recovery of minor lipid constituents, such as cholesteryl esters and phospholipids, also were studied using the above extraction and reaction sequence. Practically all conversions and extraction recoveries were over 95% on model compounds.

IMPACT/TECH TRANSFER C: The previously reported, totally automated SFE method for fat analysis in meats was optimized by freeze drying the samples prior to SFE.

OBJECTIVE D: Develop chromatographic methods for the analysis of phospholipids using evaporative light scattering detection.

PROGRESS D: Several methods reported in the literature were evaluated for the analysis of phospholipids by HPLC using evaporative light scattering detection (ELSD). Trial separations have been conducted with phospholipid standards using a Spectra Physics HPLC unit, a Varex ELSD and a Hypersil silica column, 5 micron, 200 X 4.6 mm i.d. However, these methods were not totally adequate for the separation of the four major phospholipids: phosphatidylcholine, phosphatidylethanolamine, phosphatidyl inisitol and phosphatidic acid. A solvent system was developed consisting of solvent A,

chloroform:methanol:water:ammonium hydroxide (60:34.5:5.5:0.5), and solvent B, chloroform:methanol:ammonium hydroxide (80:19.5:0.5), which proved adequate for the separation of the above four phospholipids.

A homebuilt SFC system constructed of commercial components (Isco pumps, Sedex ELSD, and a silica column) is being used to explore the utility of SFC for phospholipid analysis. This system and the HPLC unit above are being used to monitor the conversion of phospholipids to methyl esters in the NLEA SFE protocols as-well-as monitor the effectiveness of SFE for the selective extraction of specific phospholipids.

IMPACT/TECH TRANSFER D: The above described project is part of the cooperative agreement between NCAUR-ARS/USDA and the Institute of Agricultural Technology at the University of Perugia, Italy.

PUBLICATIONS:

King, J. W., F. J. Eller, J. M. Snyder, J. H. Johnson, F. K. McKeith, and C. R. Stites. 1996. Extraction of fat from ground beef for nutrient analysis using analytical supercritical fluid extraction. *J. Agric. Food Chem.* (In press).

Jackson, M. A. and J. W. King. 1996. Methanolysis of seed oils in flowing supercritical carbon dioxide. *J. Am. Oil Chem. Soc.* **73**:353–356.

Snyder, J. M., J. W. King, and M. L. Jackson. 1996. Fat content for nutritional labeling by supercritical fluid extraction and an on-line lipase catalyzed reaction. *J. Chromatogr.* (In press).

APPLICATION OF SUPERCRITICAL FLUID TECHNIQUES TO FOOD SAFETY AND NUTRIENT ANALYSIS (microextraction)

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OBJECTIVE A: Apply solid phase microextraction (SPME) for the analysis of volatiles and semivolatile compounds in foods.

PROGRESS A: Research has been completed on using the developed on-line supercritical fluid extraction (SFE) method to characterize and quantify volatile and semi-volatile compounds from meat samples. This method will appear in the *Journal of the Science of Food and Agriculture*. However, another technique is being explored due to its general applicability to food matrices, namely solid phase microextraction (SPME). Equipment ranging from a simple manual syringe injector to an automated device which will be retrofitted to our Varian Saturn ion trap GC/MS system has been purchased or recently ordered. Research plans call for this technique to be applied to several food and agricultural commodities, including samples of interest to FSIS.

IMPACT/TECH TRANSFER A: Development of the solid phase microextraction technique (SPME) is anticipated to help FSIS in solving food contamination problems. This will provide an alternative method for the detection of volatile and semivolatile compounds in foods, replacing headspace and purge & trap methodology.

PUBLICATIONS:

Snyder, J. M., J. W. King, and K. Nam. 1996. Determination of volatile and semivolatile contaminants in meat by supercritical fluid extraction/gas chromatography/mass spectrometry. *J. Sci. Food Agric.* (In press).

REFERENCE MATERIALS FOR NUTRITIONAL ANALYSIS

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OBJECTIVE A: Development and use of highly accurate methodology required to provide reference materials (RM).

PROGRESS A: Research initiated in collaboration with scientists from the National Institute of Standards and Technology (NIST) has resulted in development and availability in February of 1996 of a new Standard Reference Material 1544 "Fatty Acids and Cholesterol in a Frozen Diet Composite" using a newly developed method to determine fatty acids based upon isotope dilution mass spectrometry. Joint research by scientists from ARS, FDA and NIST has resulted in the development and availability in May 1996 of a new Standard Reference Material 1846 "Infant Formula," characterized for vitamins, minerals, proximate and calories.

IMPACT/TECH TRANSFER A: These SRM's will provide previously unavailable critically needed standards to validate food testing and analysis methodology for individual fatty acids and vitamins for use in response to nutritional labeling regulations by both FSIS and the food industry.

OBJECTIVE B: Increase knowledge and technology in matrix/analyte stability and homogeneity.

PROGRESS B: Research on long term (over four years) stability of fat soluble components in a mixed diet has been utilized to provide information for development and storage conditions of the newly available SRM (1544). A newly developed method for High Performance Liquid Chromatography analysis of niacin in infant formula/enriched flour, was utilized to determine a sampling constant (1% error due to sampling in-homogeneity) of 1–2 g for the new SRM 1846.

IMPACT/TECH TRANSFER B: This research information has allowed development of the first fresh frozen SRM (1544) characterized for nutritional organic components, and better use of the first food SRM (1846) characterized for a range of vitamins, to validate

food analysis methodology used in response to food labeling regulations by FSIS, FDA and the food industry.

OBJECTIVE C: Obtain the basis to classify foods as chemical entities and to choose representative food matrix candidate reference materials.

PROGRESS C: A scheme has been proposed to identify foods as chemical matrices defined by their position in one of nine sectors in a composition triangle with each point defined as representing 100% and the opposite side 0% of the normalized content of each of the three major components of foods; fat, carbohydrate, and protein. Foods falling in the same sector would be similar chemically and should behave in a similar analytical manner. Using this scheme will allow choice of a small number of selected food matrices for development as candidate reference materials to represent all foods. Availability of this suite of available reference materials will allow systematic judgment of applicability and validation of analytical methods over the entire range of food matrices. No additional research progress has been made on this objective during this Fiscal Year.

IMPACT/TECH TRANSFER C: The scheme to choose candidate reference material matrices representative of all foods for collaborative studies and method validation is still being considered for adoption by AOAC International.

OBJECTIVE D: Define and establish joint federal/private sector infrastructure to provide needed reference materials.

PROGRESS D: A proposal developed in collaboration with FSIS and ARS to provide a new Canned Meat SRM, characterized for components (such as total fat, protein, moisture, cholesterol, sodium, and potassium) of interest for nutritional labeling of meat and meat products has been accepted for support by NIST as a FY 97 project. Characterization of this material will be carried out through collaborative efforts between scientists from both the public and private sectors in conjunction with the Technical Division on Reference Materials (TDRM), AOAC International. A proposal to establish a national Reference Materials Committee (RMC) is under consideration by the Interagency Committee for Human Nutrition Research. This RMC would act as a focal point to evaluate needs, establish priorities and identify resources within both the public and private sectors to accelerate development of needed food based reference materials. Activity continues to establish and develop the TDRM to facilitate availability and use of reference materials within method validation, implementation and use of Official Methods of Analysis (AOAC).

IMPACT/TECH TRANSFER D: These activities will continue the progress to establish the necessary national infrastructure required to improve food/nutrition measurements.

PUBLICATIONS:

Wolf, W. R. 1996. Nutritional metrology: part-2 accuracy based measurement. *Food Testing and Analysis* **2**(1):11.

NIR SPECTROMETRY TO MEASURE NUTRIENTS

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CRIS number: **6612–44000–013**
FSIS number: **I-94–3**
CRIS Completion Date: **6–08–97**

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OBJECTIVE A: Study the usefulness on NIR technology in nutrient analysis of meat and poultry products for nutrition labeling and other regulatory samples and assess its ability to reduce the amount of hazardous waste from current laboratory methods.

PROGRESS A: Near-Infrared Proximate Analysis of Ground Beef (A collaborative study for AOAC). Plans have been formulated for a collaborative study on proximate analysis of ground beef by near infrared reflectance and transmittance as documentation for establishing an AOAC approved method. Near-infrared instrumentation, supplied from several manufactures, is widely varying in method of radiation dispersion, wavelength range utilized, presentation of radiation to a sample, and the equation applied to predict the constituent concentration. Although the optical and chemical principles of how radiation interacts within a sample are universal, the uniqueness of each manufacturer's instrument model precludes the ability to transfer equations between manufactures. For an official method on a near-infrared technique to have breadth representative of that existing in commercial instrumentation, the collaborative study is designed to encompass a multitude of instruments, each with a sufficient number of collaborators.

This study involves the evaluation of four near-infrared instruments, with 10 laboratories per instrument. The four instruments possess different combinations of the following aspects: reflectance vs. transmittance, short wavelength (850–1050 nm) vs. long wavelength (1100–2500 nm), and scanning vs. filter. They also are the four most common instruments for meat analysis. Data from each instrument model will be treated separately, as though four independent collaborative studies were underway.

In the present study the equations for fat, moisture, and protein are furnished by each instrument manufacturer. Standardization ground beef samples (N = 24) will be supplied to collaborators with proximate data for the equation slope and bias adjustment. The collaborative test samples consist of 12 independent samples without proximate data for analysis by collaborators. After equation standardization, the samples will be analyzed by each collaborator.

All instrument manufacturers have agreed to participate in the collaborative study: 1) Tecator Infratec Model 1265 (transmittance, scanning), NIRSystems Model 6500 (reflectance, scanning), Perten Instruments Model (reflectance, scanning), and Foss Meatspec (transmittance, fixed filter). Golden State Foods in Conyers, GA has agreed to supply the ground beef for the study. After the protocol is approved by AOAC, collaborators will be solicited. The project has been delayed due to my involvement in the FSIS advanced lean meat recovery (AMLR) study.

IMPACT/TECH TRANSFER A: An AOAC collaborative study with the wide variation in the combination of aspects will allow future users and manufacturers to estimate the levels of accuracy of their NIR and NIT proximate analysis. In addition, AOAC approval will allow FSIS laboratories to implement this technology for fast and accurate analysis of ground beef with no chemical waste production.

OBJECTIVE B: To determine the nutrient profiles of meat derived from advanced lean meat recovery (AMLR) systems.

PROGRESS B: ALMR systems were targeted for evaluation because the processing of beef neck bones presents an opportunity for the incorporation of additional material (i.e., blood, bone dust, "marrow" and water) into lean trimmings. Of the types of equipment used, the newest hydrau-separator requires drastic presizing of beef neck bones and its suitability for recovering meat has been questioned.

Experimental Design

1. Facilities
   7 AMLRS systems (1 Baader press, 3 Hydrau-separator, 1 Selo press, 1 Septo-matics press, and 1 Stock Protocon press).
   Control - 1 hand-deboning (whizzard knife) operations.

2. Sampling Frequency
   Random at 3 process points, 3 samples per day, and 3 times per week.

3. Sampling Duration
   3 weeks (beginning 8–26–96) (N = 567 for AMLR systems and 54 for the control).

4. Data Collection
   Nutrient: fat, moisture, protein ash, pH, color (l,a, and b; Hunter Color Meter), fatty acids, cholesterol, calcium, Hb iron, and iron.
   Instrument: Near infrared reflectance and transmittance.

5. Statistical Analysis
   Laboratory data will be statistically analyzed for differences in nutrient content of control samples vs. AMLR samples and difference among AMLR systems.

6. Spectroscopic Analysis
   Models will be developed to predict nutrient content and to determine (i.e., discriminate) when additional (i.e., blood, bone dust, "marrow", and water) material is present in lean trimmings.

All samples have been collected by FSIS Veterinary Inspector-in-Charge. Samples are being processed (i.e., ground) by FSIS Eastern Laboratory. To date we have analyzed 103 samples for proximate content, ash, color, and for fatty acids. We will report the findings of this study at the ARS/FSIS Research Workshop as-well-as the status of the AOAC collaborative study.

IMPACT/TECH TRANSFER B: In January 1995, FSIS issued a regulation to allow the ALMR technology. However, there remains concern that "marrow" is being incorporated into the final product. The results from this experiment will address this issue and possibly provide the basis for a new regulation regarding the nutrient profile of these meat products.

LOCATION ABSTRACTS

These abstracts are descriptions of the Food Safety work being conducted at each ARS location included in this report.

Albany, CA

Western Regional Research Center
800 Buchanan Street
Albany, CA 94710

Center Director
A. A. Betschart
510–559–5600

Develop intervention strategies which utilize state-of-the-art microbiological techniques and knowledge to prevent adhesion of pathogens to poultry. Identify and describe the molecular mechanisms of the attachment of pathogens to surfaces of poultry using chemical and physical techniques. Develop rapid methods for identifying pathogenic bacteria on food product surfaces using laser and thermal desorption mass spectroscopy and artificial neural network analysis. Using chemical and physical techniques, evaluate the effects of inhibitors and disinfectants on food product surfaces, and develop new disinfectants for the disinfection of meat and poultry processing water. Develop physical treatment methods for the reuse of water and brine in meat and poultry processing.

Ames, IA

National Animal Disease Center
P.O. Box 70, 2300 Dayton Avenue
Ames, IA 50010

Center Director
Thomas Walton
515–239–8201

Reduce infection and contamination from foodborne human pathogens (i.e., *Salmonella* species and pathogenic *E. coli*) on livestock and their food products, through elucidation of their pathogenesis and transmission. Delineate the immune responses of cattle and swine to zoonotic pathogens and use this knowledge to develop effective immune modulating strategies and products. Develop identification and quantification methods and control technologies to detect and reduce the prevalence of zoonotic enteric bacteria in cattle and swine to help assure the safety of animal based food products for human consumption. Improve detection and isolation methods used for diagnosis of paratuberculosis in cattle. Evaluate existing diagnostics and develop new assays for identification of cattle and cervids infected with *M. bovis* tuberculosis. Develop improved diagnostic tests and vaccines for brucellosis in cattle and wildlife.

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Develop technology to diminish and control the association with and colonization of chickens by human bacterial enteropathogens (i.e., *Salmonella* and *Campylobacter*). Minimizing consumer exposure to these enteropathogens by addressing all phases of production from the egg to the processing plant so that chickens can be delivered to processing plants with greatly reduced pathogen numbers. Develop control procedures to prevent colonization of chickens and/or turkeys with competitive exclusion cultures, and develop disinfection procedures for hatching cabinets. Prevent or reduce *Salmonella enteritidis* (SE) contamination of chicken eggs through developing better diagnostic tests, improving vaccines, determining the genetic (molecular) basis for disease production by microorganisms and identifying conditions that induce the expression of virulence genes by SE. Identify processing plant conditions, equipment and procedures that affect poultry meat safety. Develop processing innovations that enable delivery of safe, high-quality products to consumers, such as pre-evisceration treatments; spray scalding, chemical spray during feather removal, and acid/base dips. Develop and evaluate technologies such as glutamic-oxaloacetic transaminase (GOT) in thermally processed beef and poultry as indicators of end point temperature (EPT). Evaluate the significance of color changes as indicators of EPT. Determine the usefulness of near infrared (NIR) technology in nutrient analysis of meat and poultry products for nutrition labeling and assess its ability to reduce the amount of hazardous waste from current laboratory methods. Determine the nutrient profiles of meat derived from advanced lean meat recovery (ALMR) systems.

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Protect consumers from zoonotic diseases in food or water caused by parasites such as, *Cryptosporidium parvum* and *Toxoplasma gondii,* by delineating prevalence, mechanisms of parasite transmission, the infection process, parasite development and parasite-host interactions. Determine immune and genetic factors, including cytokine-regulated factors, that control responses of livestock and poultry to parasitic diseases, and identify the genes that encode such host disease resistance associated factors. Develop diagnostic and integrated control methods for livestock parasites transmissible to humans, including parasite vaccines and non-chemical anti-parasitics. Validate the use of the ELISA test as a tool for identifying trichinae-infested premises. Associate risk factors with the presence of trichinae in pigs (for on-farm control programs). Develop a low-cost assay to detect antibodies to *Toxoplasma.*

Develop simple and rapid physical-chemical procedures for detection and confirmation of antibiotic residues in animal products at levels of concern to regulatory agencies, and verify methods using incurred residues from treated animals. Develop mathematical models of the pharmacokinetics of TCDD and related compounds in beef and dairy animals. Develop methods for detection of pesticides and their metabolites in fruit, vegetables and meat in support of USDA-FSIS and USDA-AMS.

Devise nondestructive, non-invasive, and rapid systems to measure attributes of quality and safety using sensors, electronics, optics, computers, pattern recognition, artificial intelligence, and in particular, an automated, real-time system for on-line detection of unwholesome poultry carcasses (as defined by the Food Safety Inspection Service standards) in slaughter plants.

Identify mechanisms and develop control procedures to prevent inconsistent color-cooked meat temperature relationships to enable a more reliable assessment of when meat is cooked sufficiently to kill pathogens. Develop highly accurate methodology required to provide reference materials (RM) relative to measuring the nutrient content of foods and establish joint federal/private sector infrastructure to provide needed reference materials.

Clay Center, NE

U.S. Meat Animal Research Center

State Spur 18D
P.O. Box 166
Clay Center, NE 68933

Center Director
Dan Laster
402–762–4109

Develop integrated programs for efficient herd health and zoonotic disease management in beef cattle, swine, and sheep production utilizing the large herds of animals available under highly controlled experimental conditions. Evaluate and apply the knowledge gained on control of etiological agents to control zoonotic pathogens in large animal populations exposed to natural infection. Develop rapid, accurate and sensitive tests for the identification of animals infected with, and products contaminated with *Escherichia coli* O157:H7 and with *Salmonella* spp. Provide solutions to microbiological problems facing the meat industry, and respond to the needs of the Food Safety and Inspection Service. Develop methods for rapid detection of generic bacteria and specific pathogens on beef and pork carcasses. Develop carcass sanitizing procedures to reduce or eliminate generic bacteria and foodborne pathogens such as steam-vacuuming or hot water, acetic acid, lactic acid and trisodium phosphate washes. Determine various parameters that may affect removal of *E. coli* O157:H7 and other pathogens on beef carcasses. Develop a simple and representative purge sampling method to detect total aerobic bacteria and zoonotic pathogens in raw beef combos.

College Station, TX **Food Animal Protection Research Laboratory**
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409–260–9372

Develop cost effective means to prevent *Salmonella* in poultry and livestock through the use of competitive exclusion and lymphokines. Test the efficacy of the patented ARS competitive exclusion culture, CF3™, on fowl typhoid *Salmonella* (*S. gallinarum)*. Test the efficacy of CF3™ in colonization reduction of *Escherichia coli* O157:H7 in broiler chickens. Develop a model (based upon continuous-flow culture) of the poultry gastrointestinal eco-system to gain insights into the mode-of-action of competitive exclusion and provide scientific rationale for developing cultures for other food-producing animals. Develop a defined competitive exclusion culture to control *Salmonella* gut colonization in swine.

Develop monoclonal based antibody assays for detection of agrochemicals in meat and poultry products and in environmental samples. Develop, evaluate, and provide confirmatory testing of monoclonal antibody-based immunoassays useful either on-the-farm or in-the-plant (processing), and for laboratory based analysis of pesticide/drug residues in animal products and body fluids. Use molecular modeling methods to correlate binding characteristics of antibodies to the target analytes, and in the construction of immunizing haptens. Develop classical analytical methodologies (e.g., HPLC, GLC) for the analysis of pesticide/drug residues to provide confirmatory testing of the immunoassays. Develop molecularly imprinted polymers for extraction, cleanup, and detection of residues in foods. Produce food-animal tissues and body fluids containing specified levels of incurred residues of veterinary drugs, pesticides, or other chemicals for use by FSIS in the development and validation of analytical methods.

Characterize the effects of mycotoxins on animal health and productivity, and determine the extent to which selected adsorbents, therapeutic agents, and dietary modifications diminish the toxicity of mycotoxins to livestock and poultry. Test the efficacy of selected adsorbents to reduce the toxicity of mycotoxins in poultry and livestock. Determine the toxicity of fumonisins and the potential interactions between these and other mycotoxins and chemicals in poultry and livestock. Determine the significance of dietary modifications on the expression of mycotoxin toxicity.

Fargo, ND

Red River Valley Agricultural Research Center
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Center Director
Don Zimmerman
701–239–1370

Develop measures to reduce chemical residues from food products of animal origin which result from animal exposure to pesticides, antibiotics, feed additives, natural toxins or environmental pollutants. Increase the understanding of the physiological mechanisms involved in the metabolism of xenobiotics and determine the metabolic fate of these chemicals. Determine the metabolism, distribution, and excretion of β-adrenergic agonists in food producing animals. Identify and quantify residues of chlorinated dioxins and furans in beef cattle and animal feeds, in particular, forages. Devise strategies for minimizing the occurrence of these residues in meat products. Determine the metabolic disposition and excretion of dioxins in animals so that this knowledge can be used to help minimize the occurrence of residues in meat products.

Fayetteville, AR

Poultry Science Center
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Research Leader
Bill Huff
501–575–2413

Reduce the impact of poultry production problems in turkeys and broilers to ensure a wholesome product for the consumer. Determine the etiology of turkey green-liver/osteomyelitis complex (TOC), evaluate the involvement of the immune system in TOC, and develop methods to reduce the impact of TOC on turkey production. Isolate and characterize the etiological agent of proventriculitis in broilers, and develop methods to increase the intestinal strength of poultry.

Peoria, IL

National Center for Agricultural Utilization Research
1815 N. University Street
Peoria, IL 61604

Center Director
Peter Johnsen
309–681–6541

Develop supercritical fluid and conventional liquid-based size exclusion sample cleanup methods to decrease the amount of solvents necessary for residue detection and quantification. Develop SF-based sample cleanup methods using binary fluids. Develop SFE methods for mycotoxin determination in biological tissues and grains. Conduct a collaborative study to demonstrate equivalence of supercritical fluid extraction (SFE)-based methods that are similar to the protocols used for fat analysis, and their applicability to the Nutritional Labeling and Education Act (NLEA). Develop an enzymatic based hydrolysis/transesterification procedure for use in NLEA fat analysis. Develop chromatographic methods for the analysis of phospholipids using evaporative light scattering detection. Apply solid phase microextraction (SPME) for the analysis of volatiles and semivolatile compounds in foods.

Wyndmoor, PA

Eastern Regional Research Center
600 East Mermaid Lane
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Center Director
John Cherry
215–233–6595

Develop supercritical fluid extraction (SFE) instrumentation and components suitable for use in regulatory laboratories. Apply SFE for the isolation of drug and pesticide residues in eggs. Evaluate SFC for the separation and detection of trace levels of pesticide and drug residues. Residues are derivatized to make them amenable to liquid- and gas-chromatographic separation and detection at the ppb level with specialized selective and sensitive detectors. Investigate the use of automated on-line microdialysis for analyte sample preparation (analyte extraction and concentration) prior to HPLC determination. Develop state-of-the-art solvent-sparing technologies for isolation of drugs, pesticides or other residues from fresh meat, poultry, and liquid and powdered egg products in support of regulatory needs.

Investigate the safety of ionizing radiation treatments of fresh and processed meats and poultry to improve microbiological safety and shelf-life of products while preserving vitamin content. Evaluate, using modeling techniques, the delay in the onset of microbial spoilage of refrigerated chicken by gamma-irradiation. Develop a practical method to identify irradiated poultry. Characterize the effect of pH and pH history on the irradiation resistance of enterohemorrhagic *E. coli*. Determine the irradiation sensitivity of the protozoan parasites *Toxoplasma gondii* and *Cyclospora cayetanensis*.

Develop prototype equipment and processing systems to help assure food safety. Design a surface steam sterilizer to reduce microbial contamination on the surface of poultry meat without introducing significant degradation of the quality of the product.

Develop methods for detecting and controlling the potential for zoonotic disease transmission via the food supply by studying pathogen detection, behavior and characteristics. Develop knowledge of how formulation and storage factors interact to affect survival or growth of pathogenic bacteria in foods. Identify the genetic and biochemical factors that allow microorganisms to cause disease in humans. Determine how food processing and handling practices can be modified to minimize safety concerns while maximizing food quality. Specific projects include: Determine the potential of reconditioned pork processing plant water to support the growth and/or survival of various Gram negative and Gram positive bacteria. Determine the fate of *Salmonella* on pork head meat treated with alkali. Develop approaches for detachment of pathogens from meat surfaces. Develop mathematical models to estimate the behavior of foodborne pathogens in various formulations and environmental conditions. Determine the mechanism that allows foodborne pathogens to adapt to psychrotrophic growth and determine the impact of this adaptation on the organism's pathogenicity. Develop a computer software application that contains mathematical models that predict the number of *Salmonella* on poultry products as they move from the farm to the consumer.

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Poultry skin
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